Vol. XXII September, 1945 No. 5

CEREAL CHEMISTRY

Published bimonthly by the American Association of Cereal Chemists
at Prince and Lemon Sts., Lancaster, Pa.

CONTENTS

Manuscripts for publication should be sent to the Editor in Chief. Advertising rates may be secured from and subscriptions placed with the Managing Editor, Prince and Lemon Sts., Lancaster, Pa., or University Farm, St. Paul 8, Minnesota. Subscription rates, $6 per year. Foreign postage, 50 cents extra. Single copies, $1.25; foreign, $1.35.

Entered as second-class matter March 3, 1932, at the post office at Lancaster, Pa., under the act of August 24, 1912.

Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized February 16, 1924.

WALTON C. FERRIS

President and Mgr. of the National Mfg. Co.

All of us are thankful that peace has come at last, and now that our war contracts have been completed, we pledge full attention to your orders and will soon have our specialized laboratory equipment in stock for immediate shipment. The patience of our customers throughout the War period was greatly appreciated.

★ ★ ★ ★

For Test Baking—

Mixers complete
McDuffee mixer bowls
Micro Mixers
Sheeting Rolls
Moulders
Baking ovens
Baking pans
Loaf Volumeter
Photo Record Camera

For Gassing Power—

Pressuremeters
Constant Temp. Water Baths

For Moistures—

Moisture Ovens

For Maltose Determinations—

Boiling and cooling Baths

For Ash Determinations—

Ash Furnaces
Ash Crucibles
Temperature Controls

For Physical Characteristics Curves—

Recording Micro Mixer
Now called the Mixograph

CEREAL CHEMISTS' EQUIPMENT MANUFACTURER

NATIONAL MFG. COMPANY

LINCOLN, NEBRASKA

SHE EXPECTS MORE CEREAL FOODS TO BE VITAMIN ENRICHED

Taste will probably always be the largest single factor in determining the sale of a food. BUT that is no longer the whole story!

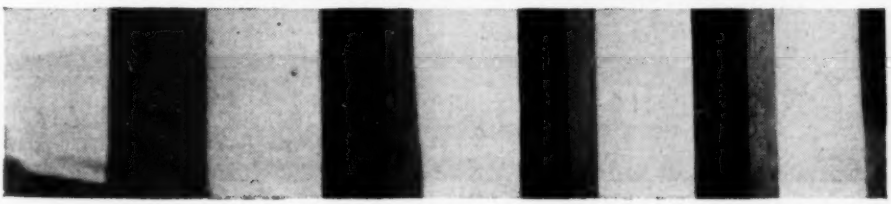
Housewives who have been studying nutrition, and men returning from the Services, have *experienced* the benefits of *improved* nutrition.

They are going to watch the labels for vitamin declarations, to pay particular attention to enriched values.

That is why the end of allocation of most vitamins has witnessed a renewal of the movement, by leaders in the food field, toward restoration and enrichment of certain foods hitherto profitable.

Are *you* sure *your* products will continue to appeal to the public as foods nutritionally well-rounded? Why not consult our Vitamin Division?

HOFFMANN-LA ROCHE, INC. • Vitamin Division, Nutley 10, N. J.



TWO "PILLARS" OF STRENGTH IN food fumigation

These two powerful Dow fumigants provide the answer to practically every food fumigating requirement.

Dow Methyl Bromide

Because of its penetrating power—unmatched by any similar product—Dow Methyl Bromide reaches the center of 140-lb. bags of grain stacked in large piles. It quickly accomplishes a complete kill of rodents and insects in all stages of development and, because it vents rapidly, permits a return to work the following morning.

Dow Chloropicrin

This well-known fumigant is especially well adapted for use in buildings not sufficiently well constructed to confine other gases. It is also widely used for rodent control, since very small quantities properly placed, will dispose of them in a hurry. Where "spot" fumigations are desired because of localized infestation, Dow Chloropicrin is thoroughly reliable.

Additional information on these two powerful answers to fumigation problems is available on request, or Dow can put you in touch with a competent industrial fumigator specializing in the food field.

DOW

THE DOW CHEMICAL COMPANY, MIDLAND, MICHIGAN

New York • Boston • Philadelphia • Washington • Cleveland • Detroit
Chicago • St. Louis • Houston • San Francisco • Los Angeles • Seattle

Millers Are Extending "ENTOLETER" Applications for Continuous Insect Control

•

"ENTOLETER" shown above handles 75 barrels per hour. Others available to handle 10, 25 or 50 barrels per hour.

More and more millers are extending "ENTOLETER" Continuous Insect Control throughout their plants. The remarkable benefits obtained through the operation of one or two "ENTOLETERS" in a mill are the most compelling reason for extending this protection to all key streams.

While all good housekeeping practices are important, the final *essential* safeguard is to make certain that no insect life is present in raw materials, in blending stocks and in the finished product before packaging. This calls for *continuous* control, applied as part of the processing operations.

Write today for bulletin, describing "ENTOLETER" applications. Includes plant layout and table of estimated costs for continuous protection. Entoleter Division, The Safety Car Heating and Lighting Company, Inc., 1153 Dixwell Avenue, New Haven 4, Connecticut.

ENTOLETER

INFESTATION DESTROYER

REG. U. S. PAT. OFF.

Imagine You

WEARING AN ASPHALT RAINCOAT!

• Probably you'd look terrible. But one thing is sure—you'd stay *dry!* Asphalt keeps out moisture. That's why we use asphalt to help keep Diamond Crystal Salt dry, and prevent caking.

Salt usually cakes when exposed to excessive humidity, because moisture condenses on salt particles, forming a thin layer of brine. Then, in dry weather, the brine evaporates and the crystals knit together.

Our research laboratory has found a number of ways to help prevent salt caking. Removal of moisture-attracting impurities, such as calcium chloride, helps. So does complete removal of fines by careful screening. Most important is to provide salt with a moisture-vapor-resistant package.

Take our Flour Salt bag, for example. It is composed of three 50-lb. and two 25-lb. sheets of kraft, laminated together with 40 lbs. of asphalt per ream. That's a lot of asphalt, and it costs us more money—but our moisture-vapor transmission tests show that this bag is well worth the extra cost. The bag is even sewn with *waxed thread* to seal the holes made by the sewing-machine needle. That's real *protection*—one reason we have been able to eliminate caking as a major problem!

WANT FREE INFORMATION ON SALT? WRITE US!

If *you* have a salt problem, write to our Director of Technical Service. He will be happy to help! Diamond Crystal Salt, Dept. M-13, St. Clair, Michigan.

DIAMOND CRYSTAL
ALBERGER PROCESS **SALT**

CONTROL

UNBIASED laboratory tests over a period of several years have conclusively proved that NATIONAL GRAIN YEAST is far superior in the matter of dough control.

And it is because of this and other outstanding qualities that thousands of progressive bakers have come to regard NATIONAL GRAIN YEAST as a primary essential in the art of baking better bread.

NATIONAL GRAIN YEAST CORPORATION

Chanin Bldg., N. Y. C. • Chicago, Ill. • Crystal Lake, Ill. • Belleville, N. J

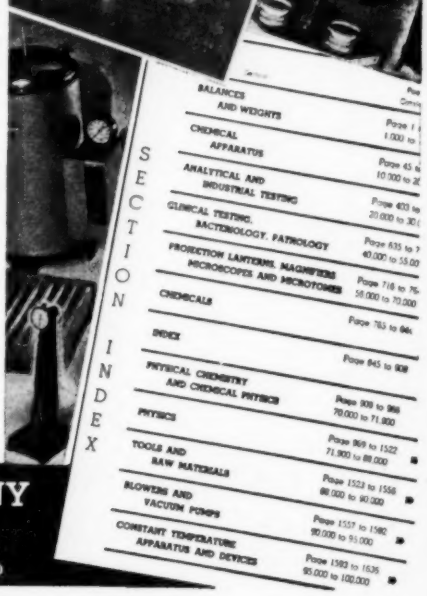
Frank J. Hale
President

OUR CATALOG

Central Scientific Company, in a little more than a third of a century, has become one of the largest manufacturers and distributors of scientific instruments and apparatus for laboratory work in major sciences, industry, and medicine.

Cenco's 1640-page J-141 Catalogue, published in 1941, is the result of the cooperation of many people, all of whom have striven to present the many devices used in scientific instruction in a manner most convenient for the user. An effort has been made to bring out the utility of each article, its flexibility in use, and details of construction that contribute to precision and durability.

The arrangement differs from the conventional of alphabetical sequence without regard to utility relationships. Logical groups have been established, within which related devices and materials are closely associated for easy comparison and selection of items. For example, under Industrial and Analytical Testing is grouped special chemical methods such as gas analysis, micro chemistry, hydrogen ion, etc., and under Industrial Testing Methods are standardization agencies, petroleum, rubber, etc. Although emphasis is just on the index, related materials will be found on near-by pages.



CENTRAL SCIENTIFIC COMPANY

SCIENTIFIC INSTRUMENTS LABORATORY APPARATUS

NEW YORK TORONTO CHICAGO BOSTON SAN FRANCISCO

CEREAL CHEMISTRY

VOL. XXII SEPTEMBER, 1945 NO. 5

STUDIES OF THE COMPOSITION OF THE WHEAT KERNEL. I. DISTRIBUTION OF ASH AND PROTEIN IN CENTER SECTIONS [1]

V. H. MORRIS, THELMA L. ALEXANDER, and ELIZABETH D. PASCOE [2]

(Received for publication May 5, 1945)

That there is a relatively wide variation in ash and protein content of individual mill stream flours has been well established by numerous analyses appearing in cereal and milling technological literature. One of the characteristics common to these analyses is a fairly consistent rise in ash and protein content parallel with the amount of break roll action. Although there is considerable disagreement as to their relative importance, this increase in ash and protein is generally attributed to two factors: (1) differential distribution in the wheat kernel and (2) incomplete separation in milling. Within the endosperm itself there is thought to be a differential distribution of ash and protein such as to account for differences in the composition of the individual mill stream flours. The evidence for this is mostly indirect, i.e., based on experience in milling. However, Cobb (1905) dissected individual wheat kernels by hand and demonstrated a relatively sharp gradient in the protein content of endosperm layers from center to bran coat. Similar studies of ash have not been made, although numerous cereal and milling technologists have suggested various values based largely on the analyses of flour streams. Estimates of several technologists on the ash content of the pure endosperm as a whole follow: Simon (1930) 0.32, Kent-Jones (1939) 0.30, and Swanson (1938) about 0.36%. In the process of breaking open the wheat kernel and separating the endosperm from the bran and germ, the cleavage is not clear-cut and it is inevitable that a certain number of bran and germ particles are also reduced to the point where they pass the flour sieves. The incorporation of a relatively small quantity of bran particles can account

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and Department of Agronomy, Ohio Agricultural Experiment Station.

[2] Senior Chemist, Junior Botanist, and Assistant Chemist, respectively, Division of Cereal Crops and Diseases at the Federal Soft Wheat Laboratory, Wooster, Ohio.

for a considerable increase in ash content, since the ash content of bran is known to be 10 to 20 times that of the lower ash flour streams.

It was thought that more accurate information on the distribution of ash and protein in the wheat kernel that might be applicable to practical milling problems could be obtained by securing and analyzing material from definite parts of the kernel. This paper reports progress in developing a technique for dissecting wheat kernels and some of the preliminary analytical data obtained in its application to different layers of endosperm.

Material and Methods

The material consisted of one variety each of the two principal classes of red winter wheat, namely, Tenmarq (hard) and Trumbull (soft). Composite samples of each class were used. These consisted

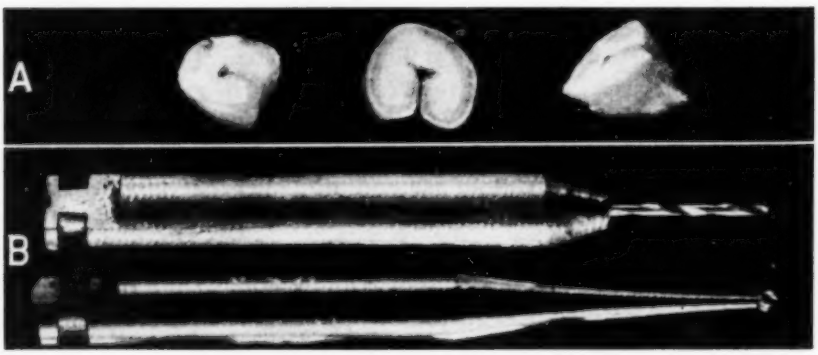


Fig. 1. A. Sections. Germ end (left), center section (middle), brush end (right). B. Drills. No. 80 wire-gauge twist drill mounted in special chuck (above). No. ½ dental burr drill (below). Magnification 5 diameters.

of a mixture of single samples collected from widely separated localities in the hard- and in the soft-wheat areas, respectively. The composite sample of Tenmarq was prepared by selecting an equal number of kernels from individual samples representing 15 different localities and five crops, 1938 to 1942, whereas that of Trumbull represented 16 localities and the five crop years 1938 to 1942.

The small size of the wheat kernel, as well as the presence of the crease, makes hand dissection difficult. For the purposes of this study it appeared that a transverse section obtained midway of the long axis of the kernel would be sufficiently representative and would be less difficult to work with than the whole kernel. Such sections were obtained by cutting the kernels transversely with a sharp scalpel at the tip of the germ and at the brush end, leaving a center section as illustrated in Figure 1 (center). The center sections of both varieties

contained, on the average, about 40% of the dry weight of the whole kernel.

The sections were tempered previous to each cutting or drilling operation by exposing to water vapor for 24 to 48 hours. The container used for tempering was a desiccator in which the usual drying agent was replaced by a dish of water.

In order to hold the center sections for dissection, they were first cemented onto standard glass microscope slides with the crease against the slide (Figure 3A). Methyl methacrylate polymer (Rohm and Haas) dissolved in chloroform was used as the cementing agent. The

Fig. 2. General view of equipment and process of dissection.

sections were first attached to the slide with a thin layer, and more of the cement was then looped over the sections. Dissection was done under a wide field binocular microscope with 6 diameters magnification. The first separations were accomplished by holding the slide edgewise and pressing the drill of the dental electric engine (Figure 2) against that portion to be removed as shown in Figure 3. The drillings were collected on a square of cellophane before transferring them to a screw-capped vial.

The endosperm was separated into four fractions ("center endosperm," "cheek endosperm," "crease endosperm," and "outer endosperm") and the bran into two ("crease bran" and "outer bran").

The appearance of the mounted sections before and after stages of dissection is illustrated in Figure 3. The "center endosperm" was secured by drilling a series of holes in the center of the endosperm with a No. 79 or 80 wire-gauge twist drill held in a special chuck (Figure 1B). The endosperm between the drill holes was next removed by pressing the drill sideways. The next fraction, "cheek endosperm," was obtained by drilling out the endosperm in the cheek of the kernel (Figure 3D) with a No. 1/2 dental burr. Approximately the same thickness of endosperm was left around the periphery next to the bran coat.

An incision was then made on each side of the crease dividing the

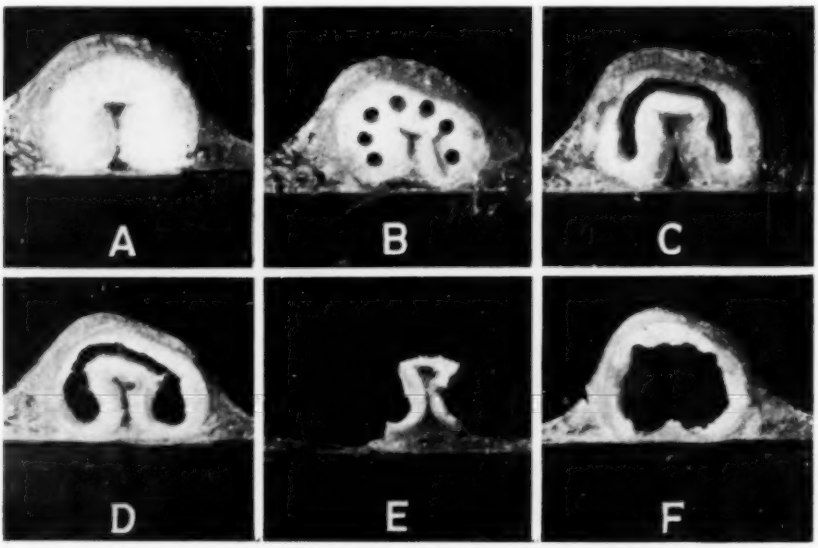


Fig. 3. Successive stages in dissection of center section. (A) mounted and ready for dissection; (B) and (C) stages in removal of center endosperm; (D) after drilling out cheek endosperm; (E) crease; and (F) outer part prior to separation of endosperm and bran. Magnification 6 diameters.

remainder of the section into two parts, "crease" and "outer" (Figure 3 E and F). The endosperm of each part was then separated from the bran by cutting almost to the aleurone layer with a sharp scalpel and lightly scraping off the endosperm. Microscopic examination of the hand-dissected bran flakes in comparison with those from a commercial mill indicated that in both instances the aleurone layer remained on the bran coat.

Some difficulty was encountered in making a clean separation between the bran and endosperm of the crease fraction because of the shape. Since one of the objectives was to obtain endosperm free of bran, the small particles were included with the bran fraction where there was any question about the presence of bran tissues (including

aleurone cells). Undissected center sections were also analyzed to serve as a check on the analysis of the various fractions, since the total of each constituent in the latter should equal that found in the former. Germ and brush ends were also analyzed. The composition of the whole wheat kernel was then calculated as either the sum or mean of the three parts (center, germ, and brush).

The fractions were dried for an hour at 135°C and weighed. In order to avoid contamination wherever possible, the endosperm fractions were used for analysis as dissected. The two bran fractions and the three sections of the kernel (center, germ, and brush ends) were ground in a small Wiley mill; since the grinding took only a few seconds, contamination from the metal of the mill does not seem likely. Protein was determined on 5 to 15 mg sub-samples by a micro-Kjeldahl procedure, replicates being required to check within 0.4% protein. The remainder of each fraction was ashed in a platinum crucible at 550°C. The ashes were weighed on a counterbalanced watch glass on the pan of an Ainsworth chainomatic balance (Model DLB) with the chain unhooked. The zero point and sensitivity were determined before and after weighing each sample, the average being used. In order to determine the approximate error of weighing the ashes, 10 aliquots of a sifted soft wheat flour were ashed and weighed. The mean was 0.355%, the maximum 0.370, the minimum 0.346, and the standard deviation 0.00072.

Results

The dissection and analytical data so far secured are presented in Tables I and II. Since this study was largely exploratory and the division into center and cheek and into crease and outer fractions was quite empirical, the quantities of dry matter, ash, and protein in certain fractions have been summed or averaged for convenience in discussing the results. The data for the center and cheek endosperm taken together are referred to as "central zone," the crease and outer endosperm as "peripheral zone," and the crease and outer bran as "total bran." The "central zone" and "peripheral zone" taken together are referred to as "total endosperm." The sum of the "total endosperm" and "total bran" constitutes the "total dissected section." The three sections of the wheat kernel—center, germ, and brush—similarly summed constitute the "whole wheat." The data thus obtained by calculation rather than by analysis are shown in Tables I and II in italics.

Dry Matter. A comparison of the average weight per 100 kernels of the center sections with the total of the several dissected fractions shows a loss of dry matter in the process of dissection of 12 and 11%

for Tenmarq and Trumbull, respectively (1.444-1.272 and 1.590-1.411 g). This probably represents fine particles lost to the air, as well as some adhering to the fine brush used in transferring endosperm drillings to the vial container. Since there was no way of ascertaining how this loss was proportioned among the several fractions, the percentage of dry matter in various fractions was calculated on the basis of the total dry matter recovered rather than on that of the undissected center sections.

Probably the most significant difference between the hard and soft

TABLE I

DRY MATTER, ASH, AND PROTEIN CONTENT OF MATERIAL DISSECTED FROM WHEAT KERNELS [1]

Item	No. kernels represented		Dry matter		Ash		Protein	
	Tenmarq	Trumbull	Tenmarq	Trumbull	Tenmarq	Trumbull	Tenmarq	Trumbull
			g/100 kernels	*g/100 kernels*	%	%	%	%
Center section								
Endosperm fraction								
Center	240	360	0.256	0.269	0.294	0.263	8.6	6.5
Cheek	260	360	.080	.171	.246	.206	9.8	7.2
Crease	151	154	.266	.234	.400	.472	12.7	10.1
Outer	151	154	.419	.437	.396	.564	14.1	11.4
Central zone			*.336*	*.440*	*.283*	*.241*	*8.9*	*6.8*
Peripheral zone			*.685*	*.671*	*.397*	*.532*	*13.6*	*10.9*
Total endosperm			*1.021*	*1.111*	*.359*	*.417*	*12.0*	*9.3*
Bran								
Crease	151	154	.116	.135	5.01	4.46	16.3	13.1
Outer	151	154	.135	.165	7.62	6.44	17.1	14.2
Total bran			*.251*	*.300*	*6.42*	*5.56*	*16.7*	*13.7*
Total dissected section			*1.272*	*1.411*	*1.56*	*1.51*	*13.0*	*10.2*
Wheat kernel								
Center section	487	484	1.444	1.590	1.30	1.44	12.1	8.4
Germ end	487	484	.992	1.484	1.92	2.06	13.3	10.9
Brush end	487	484	.922	1.162	1.51	1.63	13.4	9.9
Entire kernel			*3.358*	*4.236*	*1.54*	*1.71*	*12.8*	*9.7*

[1] Data on a 14% moisture basis.

winter wheat varieties in distribution of dry matter among the various fractions was in respect to the quantity of "cheek endosperm," the Trumbull fraction being about double that of Tenmarq in total quantity as well as in percentage. This is undoubtedly due to the difference in the shape of the kernel, the Trumbull being distinctly plumper than the Tenmarq. Since only center sections were dissected, the relative percentages of bran and endosperm as revealed in this study have no significance from a practical milling standpoint.

Ash. The number of kernels dissected represented a compromise

between two considerations: (1) the limiting factor of time and labor required for the dissection of each grain; and (2) the necessity for obtaining from the low-ash fractions a quantity of ash that could be weighed with a reasonable degree of accuracy with available facilities. The center and cheek endosperm fractions from the Tenmarq samples were dissected from 240 and 260 center sections, respectively, because preliminary studies had indicated that about this number would be necessary. For the remaining fractions, which were higher in ash, dissection was carried to completion on only 151 of the sections. The ash results were not entirely satisfactory since the sum of that found in the various fractions (0.0198 g/100 center sections) was 5% higher

TABLE II

PERCENTAGE DISTRIBUTION OF DRY MATTER, ASH, AND PROTEIN CONTENT OF MATERIAL DISSECTED FROM CENTER SECTIONS OF WHEAT KERNELS

	Dry matter		Ash		Protein	
	Proportion of total		Proportion of total		Proportion of total	
	Tenmarq	Trumbull	Tenmarq	Trumbull	Tenmarq	Trumbull
	%	%	%	%	%	%
Endosperm fraction						
Center	20.1	19.1	3.8	3.3	13.4	12.2
Cheek	6.3	12.1	1.0	1.6	4.8	8.5
Crease	20.9	16.6	5.4	5.2	20.5	16.4
Outer	32.9	31.0	8.4	11.6	35.8	34.4
Central zone	*26.4*	*31.2*	*4.8*	*5.0*	*18.2*	*20.7*
Peripheral zone	*53.8*	*47.6*	*13.8*	*16.8*	*56.3*	*50.8*
Total endosperm	*80.3*	*78.7*	*18.6*	*21.7*	*74.5*	*71.6*
Bran						
Crease	9.1	9.6	29.5	28.3	11.5	12.2
Outer	10.6	11.7	52.0	40.0	14.0	16.2
Total bran	*19.7*	*21.3*	*81.4*	*78.3*	*25.5*	*28.4*
Total dissected section	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*

than the quantity found in the center sections as a whole (0.0188 g/100 center sections). The theoretical total would be 0.0188 minus the ash contained in the 12% dry matter lost in dissecting.

In order to increase the accuracy of the ash determinations, 360 kernels were represented in the center and cheek endosperm of the Trumbull sample and the dissection was carried to completion on 154 sections. The total weight of ash recovered from the various fractions was 0.0212 g/100 sections compared with 0.0230 g found in the whole sections. The theoretical total in this instance would be 0.0230 minus the ash in the 11% dry matter lost in dissecting.

In both varieties the lowest concentration of ash was found in the cheek endosperm, this being 0.246 and 0.206% for the Tenmarq and

Trumbull samples, respectively. The ash content of the center endosperm was 0.294 and 0.263%, for the two varieties, being somewhat higher than that of the corresponding cheek fractions. In both cases the ash content of Trumbull was less than the corresponding section of Tenmarq, but the ash content of the Trumbull center endosperm was slightly higher than that of the Tenmarq cheek endosperm.

Whether the lower ash content of the cheek endosperm fraction represents a decreasing ash gradient as compared to endosperm from the center of the cheek portion of the kernel cannot be determined with certainty from this study, since a large proportion of the material included in the center fraction was obtained from that portion of the endosperm over the crease.

As previously pointed out, one of the chief points of difference between the two varieties was in the relative quantities of endosperm obtained in the cheek fractions. There was twice as much cheek endosperm by weight in Trumbull as in Tenmarq, and the cheek endosperm accounted for about 12% of the whole center section of the former but little over 6% of the latter. This larger quantity of endosperm in the central part of the soft wheat kernel, together with the distinctly lower ash content, is, perhaps, one of the reasons why relatively larger quantities of low ash patent flour can usually be obtained from soft than from hard winter wheats. Combining the data from the two endosperm fractions—the crease and the outer layer—constituting the peripheral zone, it will be noted that the Tenmarq sample contained 0.114 percentage points and the Trumbull 0.291 percentage points more ash than the average of the corresponding central zone. These represent 40 and 120% increases, respectively.

Although only two principal endosperm zones (central and peripheral) were represented in these dissections and consequently complete information was not obtained regarding possible gradients in ash concentration, the results do indicate the existence of major differences in concentration in various parts of the endosperm. The ash content of the endosperm as a whole (i.e., of the four endosperm fractions) was distinctly lower in Tenmarq (0.359%) than in Trumbull (0.417%). Although the latter figure seems unexpectedly high for a soft wheat sample, in this instance it appears logical in view of the lower whole wheat ash in Tenmarq, 1.54% compared with 1.71% for Trumbull, and the bran ash of Tenmarq, which averaged 0.86 percentage points higher than in Trumbull. It should be noted that these differences cannot be considered as conclusive evidence of fundamental differences between hard and soft red winter wheats, since each class was represented by a single variety and only from a relatively small number of years and localities.

Protein. The lowest concentrations of protein were found in the center endosperm fraction of both varieties—Tenmarq 8.6 and Trumbull 6.5%. The cheek fractions were slightly higher, Tenmarq containing 9.8 and Trumbull 7.2%. This is in distinct contrast to the relative distribution of ash.

The protein content of the peripheral zone of the endosperm (crease and outer fractions) was 13.6 and 10.9% in Tenmarq and Trumbull, respectively, as contrasted with 8.9 and 6.8 in the central zone. The protein content of the bran (crease and outer bran fractions) was 16.7 and 13.7% for the two varieties, respectively. The protein content of the endosperm as a whole (four fractions) was 12.0 and 9.3% for Tenmarq and Trumbull. In Tenmarq, the center section as a whole was almost identical in protein content with that of the total endosperm; in Trumbull, however, the center section was 0.9% lower than the endosperm. Thus, although only three zones of endosperm from the center 40% of the kernel are represented in these fractions, the results support the conclusion of Cobb (1905) based on a study of five narrower zones, namely, that the concentration of protein increases rather sharply from the center of the endosperm toward the bran layer.

It is a common observation that in the streams of flour representing additional roll action the ash content increases at a more rapid rate than the protein content. The question is often raised as to how much of the increases in ash may be due to high ash in the peripheral endosperm zones or to pulverization and subsequent incorporation of bran particles in the flour streams. It is thought that the data obtained in this study are pertinent to that question. In the Tenmarq samples the ash and protein contents of the highest fraction were each about 1.6 times those of the lowest endosperm fraction and in the Trumbull samples about 2.7 and 1.7 times for ash and protein, respectively. The average protein content of the bran fractions was only about 1.4 times that of the average endosperm in both varieties, whereas the average ash content of the bran fractions was 18 and 13 times the endosperm ash for the Tenmarq and Trumbull samples, respectively. Thus the central zone of endosperm tissue, representing about 26 to 31% of the dry matter, contained 18 to 21% of the protein but only about 5.0% of the ash; the peripheral endosperm zone, representing 48 to 54% of the dry matter, contained 51 to 56% of the protein and about 14 to 17% of the ash; the bran coat, on the other hand, representing only 20% of the dry matter, contained only 25 to 28% of the protein but 78 to 81% of the ash.

The results obtained in this study corroborate the idea previously suggested by others such as Miller (1939) that the relative rate of increase in the ash and protein content of the mill stream flours may serve

as an indication of the extent to which individual mill streams are contaminated with bran particles. To be of practical value it will be necessary to determine the range in the ash and protein content of pure endosperm, which may occur as the result of the interaction of the effects of season, location, variety, and class of wheat. Although the work herein reported is of a preliminary and exploratory nature, the results indicate the possibility of developing a microdissection technique suitable for such studies.

Summary

A microdissection technique has been developed by means of which center sections of wheat kernels have been separated into four pure endosperm and two bran fractions, and the fractions have been analyzed for ash and protein. Two classes of wheat were used, hard red winter, represented by Tenmarq, and soft red winter, represented by Trumbull. Each sample was a composite representing several crops, each grown at a number of different localities.

The lowest concentration of ash was found in the "cheek endosperm" fraction of both varieties, with the "center" endosperm fractions about 0.05% higher. The concentration of ash in the peripheral endosperm zone was considerably greater than in the cheek or center fractions, although the magnitude of the difference was not the same for both varieties. Concentrations ranged from 0.246 to 0.400% in endosperm fractions of the Tenmarq samples and from 0.206 to 0.564% in Trumbull fractions. The ash content of the total endosperm of the center section was 0.359 and 0.417% for Tenmarq and Trumbull, respectively. In the bran fractions, Tenmarq was 0.86 percentage points higher than Trumbull, 6.42 compared to 5.56%.

The pattern of distribution of protein in the various fractions was much the same as for ash except that the lowest concentration was found in the center endosperm, with the cheek fraction somewhat higher. The increase from the endosperm to the bran was much less. The protein content of the peripheral endosperm zone was 13.6 and 10.9% for Tenmarq and Trumbull, respectively, as compared with 8.9 and 6.8% in the center zone.

The rate of increase in concentration of ash and protein in the peripheral zone as compared to the central zone was about the same for both constituents. The principal point of difference in distribution was in respect to the bran coat. In these tissues the protein content was about 1.4 times that of the whole endosperm, in contrast to the ash content which was 13 to 18 times that of the endosperm.

The relatively wide difference in concentration of ash and protein in the two zones into which the endosperm was separated offers con-

siderable support to the idea of an increasing gradient in the concentration of these constituents from the center of the endosperm to the bran coat.

Literature Cited

Cobb, N. A.
1905 Universal nomenclature of wheat. Dept. Agr. New South Wales Misc. Pub. No. 539.
Kent-Jones, D. W.
1939 Modern cereal chemistry. Ed. 3, p. 301. Northern Publishing Co., Liverpool.
Miller, E. S.
1939 Ash in a new role? Northwestern Miller 200(8) Sec. 2: 4a.
Simon, E. D.
1930 The physical science of flour milling. p. 124. Northern Publishing Co., Liverpool.
Swanson, C. O.
1938 Wheat and flour quality. Burgess Publishing Co., Minneapolis, Minn.

STUDIES OF THE COMPOSITION OF THE WHEAT KERNEL. II. DISTRIBUTION OF CERTAIN INORGANIC ELEMENTS IN CENTER SECTIONS [1]

V. H. MORRIS, ELIZABETH D. PASCOE, and THELMA L. ALEXANDER [2]

(Received for publication May 5, 1945)

The extensive literature bearing on the inorganic element content of wheat and the products of milling attests to the importance of a detailed knowledge of the subject. Ample evidence that such factors as season, locality, and variety result in a wide range in mineral composition of wheat and its products is shown in numerous studies reviewed by Sullivan (1933), Beeson (1941), Booth, Carter, Jones, and Moran (1941), and Bailey (1944). One of the characteristics commonly demonstrated is a differential distribution of total ash, as well as individual inorganic elements, in bran, germ, and the various flour streams. Since the differences in flour streams may be due either to differential distribution within the endosperm or to admixture of endosperm material with varying quantities of bran and germ, interpretation of the significance of these differences from a milling standpoint is handicapped by a lack of knowledge of the general pattern of the distribution of individual elements in the wheat kernel.

---

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and Department of Agronomy, Ohio Agricultural Experiment Station.

[2] Senior Chemist, Assistant Chemist, and Junior Botanist, respectively, Division of Cereal Crops and Diseases at the Federal Soft Wheat Laboratory, Wooster, Ohio.

In extending the knowledge of the distribution of inorganic elements in the wheat kernel, one is confronted with two principal difficulties: (1) obtaining material from known parts of the kernel, and (2) development of analytical procedures suitable for analysis of fractions thus obtained. Some progress in overcoming the former obstacle is indicated in a previous paper by Morris, Alexander, and Pascoe (1945), in which data obtained by a microdissection technique are presented. These data demonstrate differences in ash and protein content of pure endosperm material from different parts of the kernel. Success attained in recent years by numerous investigators by the use of spectrographic methods in microanalytical problems has suggested the feasibility of this approach to the mineral analysis of material separated from wheat kernels. This paper describes a spectrographic procedure developed for this purpose and gives data obtained by its application to the above-mentioned fractions.

Material and Methods

The material used in this study consisted of the ash of the endosperm and the bran fractions into which center sections of kernels of the varieties Tenmarq and Trumbull were separated. The equipment and technique used in the dissection process, as well as analytical data on the distribution of ash and protein in the fractions, are reported elsewhere (Morris, Alexander, and Pascoe, 1945).

The primary prerequisites to be considered in choosing a spectrographic procedure were that it must permit the determination of most of the major, and some of the more important minor, elements in the relatively small quantities of ash available (0.5 to 5.0 mg) and with a reasonable degree of accuracy. After considerable preliminary work, a procedure was adapted from that suggested by Hess, Owens, and Reinhardt (1939). The essential feature consists in dissolving the ash in an acid solution containing a spectroscopic buffer and internal control element.

Spectrographic Procedure. Each sample of ash was brushed into a small screw-capped glass vial, and dissolved in a few drops of 1 : 1 HCl. The solution was then evaporated just to dryness to convert most of the salts to chlorides. The residue of salts was dissolved in a buffer solution added in the proportion of 4 ml for 15 mg ash. The composition of the buffer solution was: 0.57 g $Li_2SO_4 \cdot H_2O$, 11.2 g $AlCl_3 \cdot 6H_2O$, 17.8 ml conc. HCl, and 0.035 g $CdCl_2$ per 100 ml. The cadmium served as the internal control.

Seven-eighths-inch lengths of one-fourth-inch spectrographic carbons, prepared by grinding one end flat and polishing with fine sand-

paper, were purified by extraction with aqua regia, followed by washing with redistilled water until free of acid. Just prior to use, the flat surface was treated with two drops of unpurified kerosene and the electrode dried for 15 minutes at 80°C. Aliquots of 0.04 ml of the buffered sample solution were placed on four electrodes, evaporated to dryness in a forced-draft air-oven at 80°C for 15 minutes, and finally at 120°C overnight.

The arc source was the high voltage alternating current arc developed by Duffendack and Thomson (1936) operated at 2200 volts and 2.1 amperes. The electrodes were separated on the arc stand by a gap of 1.5 mm. The first pair of coated electrodes of a sample was prearced for 30 seconds with the shutter closed, and then exposed for 60 seconds. The second pair was arced in the same manner without moving the plateholder. Thus, each spectrogram was the result of duplicate exposures.

The spectrograph was a Bausch and Lomb medium quartz instrument equipped with a quartz lens which focused the image of the arc on the collimator lens. A 2-step rotating sector (ratio between steps, 11 : 1) was placed immediately in front of the slit. The slit width was 30 μ. The plateholder was moved 3 mm between spectrograms, the slit length being adjusted to produce lines a little less than 1.5 mm for each of the two steps.

The photographic plate (Eastman 33) was calibrated with a rotating stepped sector (10 steps) placed in front of the slit; the 2200 volt A.C. arc with copper electrodes was used as a light source. The plate was developed in 1 : 2 D-11 at 65°F and fixed for 5 minutes after clearing. Densities of suitable lines of the elements to be determined and the internal standard element were determined on a ARL densitometer. The log ratio of the relative intensity of the line of each element and that of the internal control element were plotted against log concentration of the element. The wave lengths of the spectral lines used were: P, 2553; K, 4044; Na, 3302; Ca, 4227; Mg, 2791; Mn, 2801; Fe, 3021; Cu, 3241; and the control element Cd, 3261. Working curves were prepared from dilutions of a standard solution containing the following concentrations of elements in grams per liter: K, 0.1257; P, 0.100; Ca, 0.010; Mg, 0.052; Mn, 0.0006; Fe, 0.002; Si, 0.002; Na, 0.00328; B, 0.0002; and Cu, 0.0004. The standard solution was 2N HCl, which contained the same concentration of spectroscopic buffers and internal control element as the previously described buffer solution. Spectrograms of a series of six dilutions containing quantities of the standard solution varying from 0.1 to 1.5 ml diluted to 4 ml with the spectroscopic buffer solution were placed on each plate. There were some

variations in the working curves from plate to plate, although for the most part the curves were slightly displaced but parallel.

Duplicate spectrograms were made of all Tenmarq fractions, except cheek endosperm where material was sufficient for a single determination only. The larger quantities of the Trumbull samples permitted triplicate spectrograms except for cheek and crease endosperm, which were made in duplicate. Because of the variation in replication, no attempt has been made to determine statistically the experimental error involved in the determination of each element. An indication of the extent of analytical error is thought to be the degree of agreement between the data representing "total dissected section" and those for the undissected section, the latter a single sample and the former a sum of six fractions. It may be noted that the figures for sodium, magnesium, and copper are distinctly higher for the dissected fractions, suggesting that the spectrographic working curves are giving results slightly, though consistently, high for these elements.

Experimental Results

The results obtained by spectrographic analysis of ash of the various fractions of endosperm, bran, and whole wheat were calculated on two bases: (1) percentage of ash, and (2) percentage of dry matter. The data are presented in Table I.

Phosphorus. The phosphorus content of the ash ranged from approximately 13 to 34%. In Tenmarq no significant differences between fractions of endosperm or between bran and endosperm are apparent, but in Trumbull the phosphorus content of the ash of the peripheral endosperm zone (crease and outer) appears to be distinctly higher than that in the central endosperm ash. The differences appear to be significant. The phosphorus content of bran ash was not different from that of endosperm ash.

Since results on a dry-matter basis are dependent not only on the concentration of the elements in the ash but also on the quantity of ash in the dry matter, it was to be expected from the distribution of total ash that the percentage of phosphorus in the peripheral endosperm zone would be considerably higher than in the central zone. This amounted to about 1.4 times the concentration in the central zone in Tenmarq and to 3.6 times in Trumbull. The phosphorus content of bran was 18 and 13 times that of endosperm for Tenmarq and Trumbull, respectively.

Potassium. The potassium content of ash of the various fractions is, within each class of wheat, probably not significantly different from that of phosphorus. They varied from about 12 to 25% in the hard

and from 19 to 43% in the soft wheat, respectively. The potassium concentration in the ash of the peripheral zone was somewhat higher than in the central zone in both varieties. The bran ash was somewhat lower than the peripheral endosperm zone but about the same as the central zone.

On a dry-matter basis the difference between the central and peripheral zones was greater than on an ash basis. On the contrary, between the endosperm and bran it was less, the bran exceeding the endosperm by 11 and 7 times only on the latter basis, as compared with 18 and 13 times on the former basis.

Sodium. In both concentration and distribution, the sodium content of the wheat kernel and its various fractions was markedly different from phosphorus and potassium. Concentrations in the ashes of Tenmarq fractions varied from about 0.1 to 1.2%, and from about 0.4 to 1.1% in Trumbull. Those fractions having the lowest total ash content (center zone) were highest in sodium, and, conversely, the tissues highest in ash (bran) were lowest in sodium content on an ash basis. The concentration of sodium in the peripheral zone ash was only about one-third that of the central zone in Tenmarq and about one-half that in Trumbull. The sodium content of bran ash was less than one-third that of the whole endosperm in the hard wheat and slightly less than three-fourths that of the whole endosperm in the soft wheat.

Although the total ash content of the two endosperm zones (central and peripheral) was markedly different in both varieties, the sodium was so nearly inversely proportional to the ash content that on a dry-matter basis the two zones were about the same in sodium, being particularly close in the soft wheat. In the bran coat, however, the decrease in sodium content of the ash was not proportionate to the increase in total ash; consequently the percentage of this element on a dry-matter basis was considerably higher in the bran than in the endosperm, amounting to about fivefold and tenfold in Tenmarq and Trumbull, respectively. In the whole kernel the concentration of sodium in the soft wheat was double that of the hard wheat.

Calcium. The concentration and distribution of calcium in the various fractions were different from the three elements previously discussed. Concentrations in the ash were uniform, within analytical error, in the endosperm fractions of the two wheats, with much lower concentrations in bran ash. In the hard wheat the bran ash contained about one-half as much calcium as the endosperm, and in the soft wheat less than one-fifth. The percentage of calcium in the whole wheat ash of the hard wheat was about twice that of the soft wheat.

TABLE I

INORGANIC ELEMENT COMPOSITION OF ENDOSPERM AND BRAN FRACTIONS FROM CENTER SECTIONS OF TWO VARIETIES OF RED WINTER WHEAT[1]

	Ash		Percentage—ash basis															
			P		K		Na		Ca		Mg		Mn		Fe		Cu	
	Ten-marq	Trum-bull	Ten-marq	Trum-bull	Ten-marq	Trum-bull	Ten-marq	Trum-bull	Ten-marq	Trum-bull	Ten-marq	Trum-bull	Ten-marq	Trum-bull	Ten-marq	Trum-bull	Ten-marq	Trum-bull
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Center section																		
Endosperm fraction																		
Center	0.342	0.306	14.3	18.4	16.8	21.8	1.11	1.09	3.6	4.7	2.7	1.4	0.065	0.109	0.46	0.43	0.67	0.31
Cheek	.286	.240	16.8	21.4	20.8	22.8	1.20	.88	4.1	4.0	5.8	2.3	.059	.103	.41	.43	.22	.16
Crease	.465	.549	13.9	27.8	25.0	32.5	.43	.66	3.6	4.2	2.4	5.0	.036	.054	.31	.45	.064	.07
Outer	.460	.656	15.7	34.3	23.7	43.0	.41	.56	3.2	3.7	2.8	4.7	.035	.068	.22	—	.059	.22
Central zone	*.329*	*.280*	*14.8*	*19.4*	*17.6*	*22.2*	*1.13*	*1.02*	*3.7*	*4.5*	*3.3*	*1.7*	*.064*	*.107*	*.45*	*.43*	*.58*	*.26*
Peripheral zone	*.462*	*.619*	*15.0*	*32.3*	*24.2*	*39.7*	*.42*	*.59*	*3.4*	*3.9*	*2.6*	*4.8*	*.035*	*.064*	*.26*	—	*.061*	*.17*
Total endosperm	*.418*	*.485*	*14.9*	*29.3*	*22.5*	*35.7*	*.60*	*.69*	*3.4*	*4.0*	*2.8*	*4.1*	*.043*	*.073*	*.31*	—	*.194*	*.19*
Bran																		
Crease	5.83	5.19	18.7	25.2	17.4	21.0	.27	.52	2.5	1.0	7.4	6.8	.31	.32	.22	.25	.045	.040
Outer	8.86	7.49	13.4	29.0	12.1	19.0	.11	.43	1.5	.6	4.5	9.4	.08	.21	.10	.35	.027	.028
Total bran	*7.46*	*6.46*	*15.3*	*27.6*	*14.0*	*19.7*	*.17*	*.46*	*1.9*	*.7*	*5.6*	*8.5*	*.16*	*.25*	*.14*	*.31*	*.033*	*.032*
Total dissected section	*1.81*	*1.76*	*15.2*	*27.9*	*15.6*	*23.1*	*.25*	*.51*	*2.2*	*1.4*	*5.0*	*7.5*	*.14*	*.21*	*.17*	—	*.063*	*.067*
Wheat kernel																		
Center section	1.51	1.68	20.1	25.0	20.9	21.5	.24	.39	2.4	.9	5.2	5.5	.16	.20	.20	.29	.046	.035
Germ end	2.23	2.39	19.5	28.2	20.2	25.3	.27	.49	2.9	1.6	5.1	6.6	.32	.36	.33	.37	.033	.037
Brush end	1.75	1.90	19.7	26.0	20.8	24.3	.20	.37	2.5	1.5	6.5	8.4	.21	.27	.19	.30	.028	.098
Entire kernel	*1.79*	*1.99*	*19.8*	*26.6*	*20.6*	*23.8*	*.24*	*.43*	*2.6*	*1.4*	*5.5*	*6.7*	*.23*	*.29*	*.25*	*.33*	*.036*	*.052*

[1] Data on a moisture free basis.

TABLE I (continued)

INORGANIC ELEMENT COMPOSITION OF ENDOSPERM AND BRAN FRACTIONS FROM CENTER SECTIONS OF TWO VARIETIES OF RED WINTER WHEAT[1]

	Dry matter per 100 kernels		Percentage—dry matter															
			P		K		Na		Ca		Mg		Mn		Fe		Cu	
	Ten-marq	Trum-bull	Ten-marq	Trum-bull	Ten-marq	Trum-bull	Ten-marq	Trum-bull	Ten-marq	Trum-bull	Ten-marq	Trum-bull	Ten-marq	Trum-bull	Ten-marq	Trum-bull	Ten-marq	Trum-bull
	g	g	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Center section																		
Endosperm fraction																		
Center	0.220	0.231	0.049	0.056	0.058	0.067	0.0038	0.0033	0.012	0.014	0.009	0.004	0.00022	0.00033	0.0016	0.0013	0.0023	0.0009
Cheek	.069	.147	.048	.051	.060	.055	.0034	.0021	.012	.010	.017	.006	.00017	.00025	.0012	.0010	.0006	.0004
Crease	.229	.201	.065	.153	.116	.178	.0020	.0036	.017	.023	.011	.028	.00017	.00030	.0014	.0025	.0003	.0004
Outer	.360	.376	.072	.225	.109	.282	.0019	.0037	.015	.024	.013	.031	.00016	.00045	.0010	—	.0003	.0014
Central zone	*.289*	*.378*	*.049*	*.054*	*.058*	*.062*	*.0037*	*.0029*	*.012*	*.013*	*.011*	*.005*	*.00021*	*.00030*	*.0015*	*.0012*	*.0019*	*.0007*
Peripheral zone	*.589*	*.577*	*.069*	*.200*	*.112*	*.246*	*.0019*	*.0037*	*.015*	*.024*	*.012*	*.030*	*.00016*	*.00039*	*.0012*	—	*.0003*	*.0011*
Total endosperm	*.878*	*.955*	*.062*	*.142*	*.094*	*.173*	*.0025*	*.0033*	*.014*	*.019*	*.012*	*.020*	*.00018*	*.00036*	*.0013*	—	*.0008*	*.0009*
Bran																		
Crease	.100	.116	1.09	1.31	1.01	1.09	.016	.027	.146	.052	.43	.35	.018	.017	.013	.013	.0026	.0021
Outer	.116	.142	1.19	2.17	1.07	1.42	.010	.032	.133	.045	.40	.70	.007	.016	.009	.026	.0024	.0021
Total bran	*.216*	*.258*	*1.14*	*1.78*	*1.05*	*1.27*	*.013*	*.030*	*.139*	*.048*	*.41*	*.55*	*.012*	*.016*	*.011*	*.020*	*.0025*	*.0021*
Total dissected section	*1.094*	*1.213*	*.28*	*.49*	*.28*	*.41*	*.0045*	*.0090*	*.039*	*.025*	*.091*	*.132*	*.0025*	*.0037*	*.0031*	—	*.0011*	*.0012*
Wheat kernel																		
Center section	1.242	1.367	.30	.42	.32	.36	.0036	.0066	.036	.015	.079	.092	.0024	.0034	.0030	.0049	.0007	.0006
Germ end	.853	1.276	.43	.67	.45	.60	.0060	.0017	.065	.038	.114	.158	.0071	.0086	.0074	.0088	.0007	.0009
Brush end	.793	.999	.34	.49	.36	.46	.0035	.0070	.044	.029	.114	.160	.0037	.0051	.0033	.0057	.0005	.0019
Entire kernel	*2.888*	*3.642*	*.35*	*.53*	*.37*	*.47*	*.0043*	*.0085*	*.047*	*.027*	*.099*	*.134*	*.0042*	*.0057*	*.0044*	*.0065*	*.0006*	*.0010*

[1] Data on a moisture free basis.

Since the calcium concentration in the ash of various fractions did not fluctuate as widely as did the total ash content, concentrations on a dry-matter basis followed in a general way the fluctuations in ash. Thus, the peripheral zones were distinctly higher in this element than the central zones, and the bran fractions much higher than the whole endosperm of both wheats. The greatest difference between the two types of wheat was in the calcium content of the bran, the hard wheat having about three times as much as the soft wheat.

Magnesium. The concentration of magnesium in the ash of the various fractions differed markedly from that of calcium in one respect —the concentration in the bran ash was double that of the endosperm in both classes of wheat. Concentrations of these elements in endosperm ashes, on the other hand, were about equal, although there was some indication that in the Trumbull sample the peripheral endosperm-zone ash was higher in magnesium than the central zone.

The contrast between the analytical results with magnesium and calcium becomes even greater when calculated on a dry-matter basis. The magnesium content of the bran was about 35 and 25 times that of endosperm as a whole in the hard and soft wheats, respectively.

Manganese. The percentage of manganese in the ash of the total endosperm was approximately one-tenth that of sodium. The distribution in various fractions (ash basis) was similar to that of sodium in that the central-zone ash contained about twice the concentration of the peripheral-zone ash but differed in that the concentration in the bran coat ash was three to four times that of the whole endosperm.

Differences in distribution of manganese become even more marked when the results are expressed on a dry-matter basis, although the endosperm fractions were relatively uniform in concentration. These results are not in accord with those of Bruere (1934). He divided wheat endosperm into three zones (central, medium, and peripheral) and found a distinct increasing gradient from central to peripheral zones. The bran coat had 66 and 44 times the concentration of the whole endosperm in Tenmarq and Trumbull, respectively. This spread in concentration between endosperm and bran layer is nearly twice that found for magnesium, 35 and 25 times, for the hard and soft wheats, respectively.

Iron. In distribution and concentration, iron seems to follow rather closely the same pattern as sodium. The ash of the outer endosperm fraction of the Trumbull sample fused in the platinum dish in ashing and was contaminated with iron in the process of removing it from the platinum crucible; consequently, the result on this sample has been omitted. The peripheral-zone ash content of Tenmarq was

about half that of the central zone and the bran coat about half that of the whole endosperm. The crease-endosperm ash of Trumbull was about the same as the central-zone fraction, however, and the bran-coat ash contained about two-thirds of the concentration of the endosperm fractions.

On a dry-matter basis the iron content of the different endosperm fractions was about equal in the hard wheat, but it was only about one-tenth of that in the bran. The data suggest that the soft wheat may contain considerably more iron than the hard wheat, the principal difference being in the bran coat.

Copper. The data on the concentration and distribution of copper are somewhat erratic. This may be due, at least in part, to the difficulty in purifying the spectrographic electrodes with respect to this element. The fact that the sum of the copper in the fractions into which the center sections were dissected was considerably greater than the analysis of the center section and to about the same degree with both varieties supports this possibility.

In general, the concentration of copper in the ash appears to follow much the same pattern as iron, and perhaps sodium and calcium. Whether the peripheral-zone ash is lower in copper than the central is not clear from these results, but the bran coat appears to be much lower than the endosperm.

When the concentrations are calculated on a dry-matter basis, however, differences in the endosperm fractions do not appear significant. The bran coat contains two or three times the concentration of copper in the endosperm.

Discussion and Conclusions

Since this study is a preliminary one and the dissection and analytical techniques are not fully developed, a detailed and completely accurate picture of the relative concentrations and distribution of the various elements within the wheat kernels should not be expected. The results do, however, suggest certain trends with respect to the central and peripheral zones; also with respect to the whole endosperm and bran.

Considering first the central and peripheral zones, the ash of the latter contained more potassium, was about equal to the central zone in phosphorus and calcium concentration, but contained a lower proportion of sodium, manganese, and copper. The results with magnesium and iron were somewhat erratic, probably due to analytical difficulties. There was a markedly greater concentration of magnesium and manganese in bran ash when compared with total endosperm; phosphorus

and potassium were about equal in concentration in endosperm and bran ashes; sodium, calcium, iron, and copper were lower in bran ash.

Since phosphorus and potassium are the major constituents in the ash, changes in total ash might be expected to be largely reflections of changes in quantities of these elements. These two elements account for 33 and 40% of the ash of the central and peripheral zones, respectively, in Tenmarq, and 40 and 70% in Trumbull. The sum of the remaining elements, sodium, calcium, magnesium, manganese, iron, and copper, accounts for only about 10% of the ash of each variety. Thus, increases in phosphorus and potassium account for a considerable part of the increase in ash of the peripheral zone, as compared with the central zone, especially in the soft wheat. The composition of bran ash differs from that of endosperm, principally in that magnesium, as well as phosphorus and potassium, is a major constituent. In concentration magnesium is about half that of potassium. The phosphorus content in this fraction is somewhat higher than potassium—a contrast to the situation found in the endosperm. The sum of the remainder of the elements determined accounts for only about 2% of the total bran ash.

Since the hard and soft winter-wheat regions were represented by only a single variety in this study, definite conclusions cannot be drawn regarding fundamental differences in these wheats. Furthermore, the endosperm ash content of Trumbull (0.485) seems rather high to be typical of the eastern soft wheat region. It is of interest to note, however, that, on a percentage basis, the endosperm of the soft wheat contained a higher concentration of each of the elements determined than did that of the hard wheat, although the difference in calcium was small and that of copper probably not significant.

Summary

A spectrographic procedure is described for the quantitative analysis of small quantities of wheat ash for the major elements, phosphorus, potassium, sodium, calcium, and magnesium, as well as the three minor elements, manganese, iron, and copper. The method was applied to the analysis of the ashes of four pure endosperm and two bran fractions dissected from center sections of two wheat varieties. Two classes of wheat, hard red winter (Tenmarq) and soft red winter (Trumbull), were represented. For purposes of discussion, the data on the various fractions were combined into two endosperm zones, central and peripheral, and the bran coat.

On an ash basis, potassium was higher in concentration in the peripheral than in the central zone; phosphorus and calcium about the

same in the two zones; sodium, manganese, and copper were lower in the peripheral zone. The trends of magnesium and iron were uncertain because of inconsistent results. Comparing concentrations in the endosperm as a whole ("total endosperm") with those in the bran coat, magnesium and manganese were higher in bran ash, and phosphorus and potassium were about the same in the two tissues; sodium, calcium, iron, and copper were higher in the endosperm.

On a dry-matter basis, the peripheral zone contained higher concentrations of phosphorus, potassium, calcium, and probably magnesium than did the central zone. Results with the remaining elements were inconsistent. The relatively higher ash content of bran as compared with endosperm is reflected by the fact that concentrations of all the elements were higher in the former.

Of the relatively large differences in total ash shown to exist between the central- and peripheral-endosperm zones, the two major ash constituents—phosphorus and potassium—account for a considerable part of the higher ash in the latter zone. The composition of the bran resembled that of endosperm in that both phosphorus and potassium were major constituents. It differed, however, in that magnesium also was a major constituent, the concentration equalling about half that of potassium.

Literature Cited

Bailey, C. H.
1944 The constituents of wheat and wheat products. pp. 205–239. Reinhold Publishing Co., New York.

Beeson, K. C.
1941 The mineral composition of crops with particular reference to the soils in which they were grown. A review and compilation. U. S. Dept. Agr. Misc. Pub. No. 369.

Booth, R. G., Carter, R. H., Jones, C. R., and Moran, T.
1941 The nation's food. 2. Cereals as food. Chemistry of wheat and wheat products. Chem. and Ind. **60**: 903–908.

Bruere, P.
1934 Taux et repartition du manganese dans le grain de blé. Compt. rend. **198**: 504–506.

Duffendack, O. S., and Thomson, K. B.
1936 The developments in the quantitative analysis of solutions by spectroscopic means. Proc. Am. Soc. Testing Materials **36**(II): 301–309.

Hess, T. M., Owens, J. S., and Reinhardt, L. G.
1939 Analysis of organic materials for traces of metallic impurities. Ind. Eng. Chem. (Anal. Ed.) **11**: 646–649.

Morris, V. H., Alexander, T. L., and Pascoe, E. D.
1945 Studies of the composition of the wheat kernel. I. Distribution of ash and protein in center sections. Cereal Chem. **22**: 351–361.

Sullivan, B.
1933 The inorganic constituents of wheat and flour. Cereal Chem. **10**: 503–514.

PRELIMINARY STUDIES WITH THE EXTENSOGRAPH [1]

PAUL P. MERRITT and C. H. BAILEY

Division of Agricultural Biochemistry,
University of Minnesota, St. Paul, Minnesota

(Presented at the Annual Meeting, May, 1944; received for publication June 30, 1944)

The physical properties of doughs, both leavened and unleavened, have been observed and discussed by a great many investigators. It has long been known that the physical properties of leavened doughs are progressively altered as fermentation progresses, partly as a consequence of the interaction of the materials in the dough and partly as a result of the mechanical treatment it receives.

Bailey (1940) and Swanson (1943) have reviewed and discussed the more important contributions to the literature on this subject.

In the present investigation the effects of several factors on the physical properties of doughs prepared from flours of varying strength have been measured with the Brabender extensograph. Studies were made of the effect of variations in the technique of the test and of the influence of oxidizing agents, reducing agents, fats, and nonfat milk solids on the characteristics of the extensograph curve. An expression was formulated which may prove useful in predicting the oxidizing requirements of doughs as satisfied with potassium bromate.

Materials and Methods

The extensograph used in making the measurements was essentially the same as that described by Bailey (1940), with the exception that the dough hook was made to travel vertically rather than circumferentially. It has built-in provisions for controlling some of the variables in the test. These include a device for working up the dough, a molder, a constant-temperature incubator, a constant-speed traveling hook for engaging and stretching a test piece of dough, and an electrically operated kymograph with an inking stylus for continuously recording stresses in the portion of dough under test, as well as the distance that it is extended.

Of the measurements that may be made from the curve traced during the extension of the dough, the most important ones are the length of the base line, which records extensibility (E); the maximum height, which records resistance to extension (H); and the area enclosed by the curve and the base line, which is a function of the work done on

[1] Paper No. 2179, Scientific Series, Minnesota Agricultural Experiment Station, condensed from a thesis presented by Paul P. Merritt to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1944.

the dough during extension (A). Various relationships of these characteristics may be calculated. A typical extensogram with the dimensions that are measured is shown in Figure 1.

Because of the irregular manner in which the extended cylinder of dough broke, the only dimensions that will be considered in this paper are the maximum resistance to extension (H), the extensibility at the time of maximum resistance (E), and the area enclosed by the base line and the extensogram at the time of maximum resistance (A_1).

In the studies conducted by Munz and Brabender (1940), the extensograph was adjusted so that a 125 g load on the balance corresponded to 100 units of resistance, as recorded on the extensograph paper. As they worked with many weak flours it seemed desirable, in

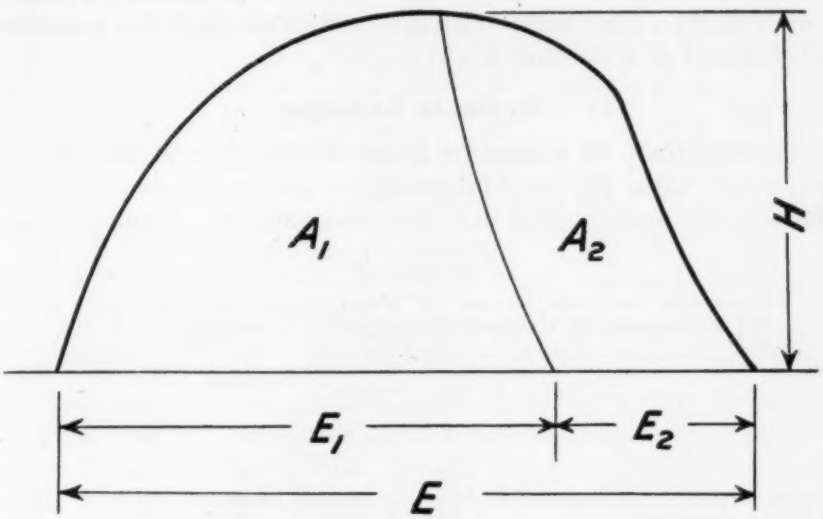


Fig. 1. Extensogram showing the dimensions that were measured.

the present study, to use a higher load setting, and the instrument was adjusted to record 60 units of resistance per 100 g load. The velocity of the kymograph paper was found to be 0.65 cm per second, and that of the dough hook 1.41 cm per second.

Instruments employed in preparing doughs for testing with the extensograph were the Hobart-Swanson dough mixer and the farinograph.

Three commercially milled flours were used, a strong flour (capable of producing loaves of relatively large loaf volume, Table V), a medium-strong flour, and a weak flour containing 12.5, 10.6, and 8.6% of crude protein ($N \times 5.7$), respectively.

Unleavened doughs, unless otherwise specified, were used in the

extensograph tests. They were mixed in a farinograph that had been freed of surface oxides on the parts coming in contact with the dough. This was done by mixing a blank dough for 30 minutes, followed immediately by the test dough, as directed by Stamberg and Merritt (1941). A 480-g dough was mixed until minimum mobility was reached, employing an absorption that resulted in a dough of 500 consistency units at minimum mobility. The dough was scaled into three portions of 150 g, each of which was rounded-up with 20 revolutions of the rounder, then molded, placed in a dough holder, incubated for 45 minutes at 30°C, and an extensogram traced. Following extension, the dough was rerounded, remolded, and again incubated for a second rest period of 45 minutes, following which it was extended a second time. The dough was treated as before, allowed a third 45-minute rest period, and extended a third time. Unless otherwise specified, this procedure was followed as a standard practice.

Studies on Technique

Replicability. As a measure of the replicability of the extensograph test, using the standard procedure, the strong flour was used since doughs made with it were the most variable. Standard errors

TABLE I

MEANS AND STANDARD ERRORS OF MEASUREMENTS OF DIMENSIONS OF EXTENSOGRAMS FOR UNLEAVENED DOUGH MADE FROM STRONG FLOUR

	Dimensions[1]				
	E	E_1	H	A	A_1
	cm	cm	cm	cm^2	cm^2
Means	14.6	10.9	630.0	110.0	75.1
S.E. of a single test	1.00	0.44	79.4	11.5	8.9
S.E. of a mean of three tests	0.58	0.25	45.8	6.6	5.2
S.E. of a difference between two such means	0.82	0.36	64.8	9.4	7.3

[1] Based on 108 extensograms.
E = Extensibility; measured from the moment it is first stretched to the final break.
E_1 = Extensibility at the time of maximum resistance.
H = Maximum resistance to extension.
A = Total area under the curve.
A_1 = Area enclosed by the curve and the base line at the time of maximum resistance.

(S.E.) of the test with the most variable flour should be maximum values and hence safer to use as measures of significance when applied to the results of tests with less variable flours. Table I lists the mean values and standard errors of the dimensions of extensograms secured in the test of replicability.

Effect of Time and Reworking. The data showing the combined effect of rest time and the reworking and repeated extension of the test

piece of dough on the extensograms of doughs prepared from the strong, medium, and weak flours are recorded in Table II. The extensibility, E_1, and the curve area, A_1, decreased with rest time and reworking. Resistance to extension, H, was increased with the stronger flours; with the weak flour, resistance to extension was at a maximum after the second rest period.

TABLE II

EFFECT OF TIME AND REWORKING ON EXTENSOGRAM DIMENSIONS

Extensogram dimensions [1]	Flours								
	Strong			Medium			Weak		
	Rest time, min			Rest time, min			Rest time, min		
	45	90	135	45	90	135	45	90	135
E_1 (cm)	13.0	8.9	7.6	12.8	9.7	8.4	7.3	5.9	5.5
H (eu)	618	952	982	440	708	795	488	582	543
A_1 (cm^2)	91.8	85.8	76.8	66.4	74.4	69.7	40.5	37.3	31.7

[1] E_1 = Extensibility at the time of maximum resistance.
H = Maximum resistance to extension.
A_1 = Area enclosed by the curve and the base line at the time of maximum resistance.

Effect of Working Once vs. Repeated Working of Dough Pieces. The manual of instructions supplied with the extensograph by the manufacturers recommends repeated tests of the same piece of dough after rest periods of 45 minutes each, and a series of experiments was planned to test the advisability of this procedure. One unleavened dough was tested by the standard procedure already described. Another unleavened dough was divided into three portions and tested, one after a 45-minute rest time, a second after an uninterrupted 90-minute rest time, and a third piece after an uninterrupted 135-minute rest time. The same tests were made with leavened doughs containing 3% yeast. The data are recorded in Table III.

Extensograms made with unleavened doughs after rest times of 90 and 135 minutes with only one working of the dough were similar. Extensibility, resistance to extension, and curve area tended to change very little upon increasing the rest times, but with repeated testing of the same piece of dough, extensibility and curve area decreased and the resistance to extension increased with an increase in the rest time. Essentially similar results were obtained with leavened doughs. These results justify the technique recommended by the manufacturer. The use of leavened doughs for extensograph testing is not recom-

TABLE III

EFFECT OF WORKING DOUGHS ONLY ONCE AFTER 45, 90, AND 135 MINUTE REST TIMES AS COMPARED WITH TESTING THE SAME DOUGH REPEATEDLY AFTER REWORKING

Flour	Extensogram dimensions [1]	Units	Rest time, minutes				
			45	90	135	90	135
			Reworked at 45-min intervals			Worked once	
UNLEAVENED							
Strong	E_1	cm	14.6	9.6	8.7	15.2	15.8
	H	eu	530	717	789	475	432
	A_1	cm²	87.2	72.5	72.5	82.1	80.4
Medium	E_1	cm	14.8	11.5	9.9	15.3	15.0
	H	eu	369	503	543	320	310
	A_1	cm²	64.4	64.3	60.6	59.2	56.2
Weak	E_1	cm	7.8	6.3	5.4	8.8	9.5
	H	eu	428	588	515	380	392
	A_1	cm²	40.0	38.3	29.3	41.5	46.3
LEAVENED							
Strong	E_1	cm	13.2	9.2	8.1	8.0	8.0
	H	eu	372	414	397	310	295
	A_1	cm²	54.6	38.2	29.1	25.6	26.1
Medium	E_1	cm	12.8	8.7	7.3	9.0	7.7
	H	eu	254	306	343	266	242
	A_1	cm²	38.1	27.6	23.5	27.6	19.2
Weak	E_1	cm	6.8	4.8	4.3	7.2	7.3
	H	eu	293	315	296	214	226
	A_1	cm²	23.0	15.5	12.8	18.9	20.0

[1] E_1 = Extensibility at the time of maximum resistance.
H = Maximum resistance to extension.
A_1 = Area enclosed by the curve and the base line at the time of maximum resistance.

mended because of increased difficulty of handling. Dimensions of extensograms were greatly reduced in magnitude, also, although the same trends in changes were noted as when reworked unleavened doughs were used.

Effect of Various Substances on Dimensions of Extensograms

Oxidizing Agents. Potassium bromate in dosages of 1, 2, and 3 mg per 100 g of flour was added to the unleavened doughs which were tested with the extensograph by the standard procedure already described. The data from these tests are recorded in Table IV. The addition of potassium bromate decreased extensibility, resistance to

extension, and area of the extensogram. Generally, the effect was more pronounced after the dough had been reworked twice.

The loaf volumes of bread baked from leavened doughs containing varying quantities of potassium bromate are shown by the data recorded in Table V. In these baking tests, the doughs were fermented 45 minutes, then molded and proofed 55 minutes to correspond to an extensograph-tested unleavened dough rested for 45 minutes. To correspond to doughs tested with the extensograph after one or two previous extensions and reworkings, the fermented doughs were punched with a dough sheeter after 45 and 90 minutes.

TABLE IV

EFFECT OF POTASSIUM BROMATE ON EXTENSOGRAM DIMENSIONS OF UNLEAVENED DOUGHS

Extensogram dimensions [1]	$KBrO_3$ per 100 g flour	Flours								
		Strong			Medium			Weak		
		Rest time, min			Rest time, min			Rest time, min		
		45	90	135	45	90	135	45	90	135
	mg	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*
E_1	0	9.6	7.3	6.7	12.8	9.7	8.4	7.3	5.9	5.5
	1	10.2	7.2	6.1	12.8	8.8	8.2	7.1	5.6	5.3
	2	9.3	6.6	6.0	12.3	8.3	7.4	5.7	5.0	4.6
	3	9.7	6.9	5.8	11.4	7.9	7.0	6.3	5.1	4.6
		eu	*eu*	*eu*	*eu*	*eu*	*eu*	*eu*	*eu*	*eu*
H	0	398	720	737	440	708	795	488	582	543
	1	443	763	768	412	642	680	482	607	547
	2	408	697	669	405	642	620	427	519	440
	3	325	615	659	415	652	705	453	548	498
		cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2
A_1	0	45.6	53.6	46.8	66.4	74.4	69.7	40.5	37.3	31.7
	1	52.4	56.1	45.8	62.9	61.7	58.8	39.9	36.3	29.8
	2	43.3	45.5	38.5	57.0	57.1	45.6	27.5	27.0	20.4
	3	38.1	44.2	38.1	55.4	58.9	49.0	32.8	29.7	23.7

[1] E_1 = Extensibility at the time of maximum resistance.
H = Maximum resistance to extension.
A_1 = Area enclosed by the curve and the base line at the time of maximum resistance.

The strong flour showed the maximum increase in loaf volume after 45 minutes fermentation with 3 mg of potassium bromate, but with the longer fermentations 1 mg of potassium bromate gave a maximum response in loaf volume. The medium strength flour gave about the same positive response to all dosages of potassium bromate after 45 minutes of fermentation, but showed slight decrease in loaf volume as compared with the control after 90 minutes of fermentation and considerable decrease after 135 minutes, particularly with the 2 and 3 mg

dosages. The loaf volumes for the weak flour decreased with both time and increasing quantities of the oxidizing agent.

Potassium iodate is sometimes used in doughs as an oxidizing agent. It is known to be more effective than potassium bromate when used in the same quantities. Varying quantities were added to unleavened doughs and extensograms made by the standard procedure. The data are recorded in Table VI.

Extensibility of the doughs was increased with increasing dosages of potassium iodate, especially after the doughs had been extended once or twice previously. In this respect, the action of potassium iodate differed from that of potassium bromate. Resistance to extension was

TABLE V

EFFECT OF POTASSIUM BROMATE ON LOAF VOLUME

Flours	$KBrO_3$ per 100 g flour	Loaf volume after fermentation—min			
		45	90	135	Mean
	mg	*cc*	*cc*	*cc*	*cc*
Strong	0	600	743	750	698
	1	655	778	775	736
	2	672	775	762	736
	3	692	772	688	717
Mean		655	767	744	722
Medium	0	603	688	610	634
	1	637	677	603	639
	2	632	662	563	619
	3	635	667	573	625
Mean		627	674	587	629
Weak	0	473	460	438	457
	1	470	448	418	445
	2	468	442	408	439
	3	460	442	412	438
Mean		468	448	419	445

decreased as the quantity of the iodate was increased and at a greater rate than the corresponding decrease produced with the bromate. The addition of 1 mg of potassium iodate produced a lower value for resistance to extension, *H*, than did 3 mg of potassium bromate. The area of the extensogram was decreased as the quantity of potassium iodate was increased, particularly so when the doughs were extended for the first time. With dosages of 0.25 mg of potassium iodate per 100 g of flour, the curve areas decreased as the tests were repeated. The reverse was true with a dosage of 2 mg except in the case of the weak flour. This behavior is considerably different from that when bromate was used, and may indicate that iodate has less tendency to make doughs "bucky" than does bromate.

TABLE VI

EFFECT OF POTASSIUM IODATE ON EXTENSOGRAM DIMENSIONS OF UNLEAVENED DOUGHS

Extensogram dimensions [1]	KIO_3 per 100 g flour	Flours								
		Strong			Medium			Weak		
		Rest time, min			Rest time, min			Rest time, min		
		45	90	135	45	90	135	45	90	135
	mg	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*
E_1	0.00	12.7	9.0	7.8	12.4	8.7	7.7	6.0	5.5	5.1
	0.25	9.3	7.4	6.7	12.5	8.5	7.7	5.8	5.1	4.8
	0.50	11.0	8.4	7.1	11.7	8.4	7.7	6.3	5.4	5.2
	1.00	11.3	8.5	7.6	11.0	9.7	8.7	5.8	6.0	5.8
	2.00	9.1	8.6	8.3	8.1	7.6	7.9	6.2	5.6	5.5
		eu	*eu*	*eu*	*eu*	*eu*	*eu*	*eu*	*eu*	*eu*
H	0.00	611	845	854	455	652	685	498	544	460
	0.25	716	902	839	403	621	661	409	499	427
	0.50	623	881	840	443	710	688	463	540	487
	1.00	295	597	712	359	607	728	359	408	412
	2.00	204	372	456	191	276	277	345	363	343
		cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2
A_1	0.00	86.0	79.0	66.4	65.3	60.6	53.2	32.8	30.4	24.4
	0.25	70.4	65.8	54.0	58.4	57.2	52.9	26.6	26.8	21.1
	0.50	76.8	72.9	58.8	62.1	62.7	55.4	32.9	31.0	26.1
	1.00	42.4	55.7	55.3	50.5	66.3	66.4	23.0	28.0	26.6
	2.00	24.4	37.3	43.0	20.7	25.7	26.6	24.5	22.3	20.8

[1] E_1 = Extensibility at the time of maximum resistance.
H = Maximum resistance to extension.
A_1 = Area enclosed by the curve and the base line at the time of maximum resistance.

Reducing Agents. Sodium sulfite, as well as other reagents containing sulfur in a reduced form, was observed by Blish (1934) to counteract or reduce the effects of overtreatment of doughs with oxidizing agents and to be effective when applied to the whole grain cereal, to the flour, or to the dough made with the flour. Sodium sulfite was added in various dosages to unleavened doughs and the resulting extensograph data are recorded in Table VII. The quantity of sodium sulfite that would approximately counteract the oxidizing power of 1 mg of potassium bromate was calculated to be 2.27 mg.

Extensibility of the doughs at each rest time increased as the quantity of reducing agent was increased. The decrease in extensibility with an increase in rest time was much less than the corresponding change which occurred when potassium bromate was used. Resistance to extension decreased with an increase of sodium sulfite and to a greater extent with the medium-strength and weak flours than with the strong flour. For all three flours the extensogram area decreased as the

quantity of sulfite was increased; this change was much less than those noted with the two oxidizing agents.

Data showing the effect of sodium sulfite on loaf volumes are recorded in Table VIII. These loaf volumes were secured in the same way as those previously described when potassium bromate was used. Sodium sulfite had little effect on the loaf volume of the strong flour, but slight increases in volume were obtained with the medium and weak flours, especially with the longest fermentation. This might be

TABLE VII

EFFECT OF SODIUM SULFITE ON EXTENSOGRAM DIMENSIONS OF UNLEAVENED DOUGHS

Extensogram dimensions[1]	Na_2SO_3 per 100 g flour	Flours								
		Strong			Medium			Weak		
		Rest time, min			Rest time, min			Rest time, min		
		45	90	135	45	90	135	45	90	135
	mg	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*
E_1	0.00	12.7	9.0	7.8	12.4	8.7	7.7	6.0	5.5	5.1
	2.27	13.1	9.4	8.2	13.3	10.2	8.2	6.9	5.5	5.4
	4.54	13.8	9.4	8.5	13.6	10.7	9.6	8.0	6.2	5.5
	6.81	13.5	9.7	8.5	14.4	12.0	10.7	9.9	6.6	6.2
		cu	*cu*	*cu*	*cu*	*cu*	*cu*	*cu*	*cu*	*cu*
H	0.00	611	845	854	455	652	685	498	544	460
	2.27	615	862	850	458	708	740	487	607	567
	4.54	511	830	862	331	558	625	383	572	536
	6.81	537	796	815	235	407	476	190	377	409
		cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2
A_1	0.00	86.0	79.0	66.4	65.3	60.6	53.2	32.8	30.4	24.4
	2.27	91.9	82.6	76.1	71.5	78.9	64.2	39.4	35.1	30.9
	4.54	82.2	82.7	74.3	54.6	67.7	64.3	37.0	37.6	30.8
	6.81	83.5	81.0	71.0	43.5	55.3	57.8	25.3	28.7	28.0

[1] E_1 = Extensibility at the time of maximum resistance.
H = Maximum resistance to extension.
A_1 = Area enclosed by the curve and the base line at the time of maximum resistance.

expected since the weaker flours showed little or no positive response to potassium bromate.

Since potassium bromate and potassium iodate did not show identical effects at any of the dosages tried, it was of interest to measure the effect of another sulfur-containing reducing agent. The extensograph data secured with the use of varying quantities of sodium thiosulfate are recorded in Table IX.

Extensibility increased as the quantity of sodium thiosulfate was increased when the strong flour was tested after one or two rest periods; however, after two previous tests, the extensibility decreased with an

TABLE VIII

Effect of Sodium Sulfite on Loaf Volume

Flours	Na_2SO_3 per 100 g flour	Loaf volume after fermentation—min			
		45	90	135	Mean
	mg	*cc*	*cc*	*cc*	*cc*
Strong	0.00	615	765	782	721
	2.27	615	752	750	706
	4.54	625	760	755	713
	6.81	625	735	768	709
	Mean	620	753	764	712
Medium	0.00	612	690	618	640
	2.27	610	715	625	650
	4.54	608	658	635	634
	6.81	590	640	635	622
	Mean	605	676	626	636
Weak	0.00	485	458	435	459
	2.27	480	465	448	464
	4.54	480	470	450	467
	6.81	482	480	458	473
	Mean	482	468	448	466

TABLE IX

Effect of Sodium Thiosulfate on Extensogram Dimensions of Unleavened Doughs

Extensogram dimensions [1]	$Na_2S_2O_3$ per 100 g flour	Flours								
		Strong			Medium			Weak		
		Rest time, min			Rest time, min			Rest time, min		
		45	90	135	45	90	135	45	90	135
	mg	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*
E_1	0.00	12.7	9.0	7.8	12.4	8.7	7.7	6.0	5.5	5.1
	1.42	12.0	8.7	8.5	11.9	8.5	7.5	6.2	4.9	4.6
	2.84	13.5	8.9	7.6	13.6	9.3	8.5	7.2	5.8	4.8
	4.26	13.5	9.1	7.3	13.1	9.4	8.1	6.7	5.4	5.1
		cu	*cu*	*cu*	*cu*	*cu*	*cu*	*cu*	*cu*	*cu*
H	0.00	611	845	854	455	652	685	498	544	460
	1.42	543	729	729	388	607	609	408	523	433
	2.84	475	759	757	347	583	669	357	477	459
	4.26	423	735	746	319	538	589	330	431	419
		cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2
A_1	0.00	86.0	79.0	66.4	65.3	60.6	53.2	32.8	30.4	24.4
	1.42	74.2	67.0	53.7	54.1	56.7	48.7	28.5	27.0	21.4
	2.84	75.9	74.2	59.1	57.7	61.5	60.9	30.9	29.8	23.7
	4.26	68.7	71.2	56.6	51.2	56.2	51.4	26.7	25.6	21.9

[1] E_1 = Extensibility at the time of maximum resistance.
H = Maximum resistance to extension.
A_1 = Area enclosed by the curve and the base line at the time of maximum resistance.

increase in the quantity of this reagent. With the weaker flours, the addition of the reducing agent tended to increase the extensibility only very slightly. This might be expected since the strong flour was shown to have a considerable bromate requirement and a reducing agent should have an opposite effect to that of bromate. The weaker flours, having

TABLE X

EFFECT OF VARYING QUANTITIES OF POTASSIUM BROMATE AND SODIUM SULFITE ON EXTENSOGRAM DIMENSIONS

Extensogram dimensions [1]	$KBrO_3$ per 100 g flour	Na_2SO_3 per 100 g flour	Flours								
			Strong			Medium			Weak		
			Rest time, min			Rest time, min			Rest time, min		
			45	90	135	45	90	135	45	90	135
	mg	*mg*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*
E_1	0	0.00	12.7	9.0	7.8	12.4	8.7	7.7	6.0	5.5	5.1
	1	2.27	10.8	8.0	7.2	10.1	7.5	6.4	5.7	5.0	4.8
	3	2.27	12.3	8.5	7.2	13.3	9.5	8.3	6.3	5.2	4.8
	2	4.54	12.0	8.5	7.4	12.1	9.1	7.3	6.1	5.1	4.7
	3	4.54	11.3	8.0	7.1	13.8	9.6	8.6	7.1	5.2	4.9
	1	6.81	12.8	8.7	7.5	14.3	10.8	8.9	9.6	6.2	5.8
	2	6.81	12.4	8.6	7.5	12.7	8.7	7.7	6.4	5.2	4.8
	3	6.81	10.5	7.7	6.8	11.8	8.3	7.1	6.4	5.2	4.9
			cu	*cu*	*cu*	*cu*	*cu*	*cu*	*cu*	*cu*	*cu*
H	0	0.00	611	845	854	455	652	685	498	544	460
	1	2.27	729	859	747	513	723	631	415	451	411
	3	2.27	552	741	694	395	559	555	466	483	451
	2	4.54	591	793	703	437	567	547	437	488	439
	3	4.54	571	720	684	358	526	460	399	495	425
	1	6.81	567	789	744	260	415	460	237	373	355
	2	6.81	585	785	739	435	616	598	460	498	467
	3	6.81	690	835	720	473	726	672	450	536	483
			cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2
A_1	0	0.00	86.0	79.0	66.4	65.3	60.6	53.2	32.8	30.4	24.4
	1	2.27	84.7	68.6	51.8	61.0	57.4	42.7	25.9	22.8	20.2
	3	2.27	76.9	65.9	50.9	61.8	58.5	49.2	31.9	26.6	22.6
	2	4.54	79.6	68.0	51.6	61.6	55.3	43.0	30.7	26.5	20.9
	3	4.54	70.9	59.3	42.0	58.1	58.0	42.9	32.5	27.2	21.3
	1	6.81	81.8	71.2	57.9	45.3	51.9	46.3	27.9	26.5	23.0
	2	6.81	81.2	70.2	56.1	63.2	59.0	48.9	32.7	27.4	22.7
	3	6.81	80.1	65.0	48.3	65.1	63.6	47.9	34.6	29.2	24.6

[1] E_1 = Extensibility at the time of maximum resistance.
H = Maximum resistance to extension.
A_1 = Area enclosed by the curve and the base line at the time of maximum resistance.

little or no bromate requirement, would naturally be only slightly affected by reducing agents in the dosages used. It is reasonable to expect that larger quantities of reducing agent would have had an effect opposite to that of bromate on the weaker flours.

Resistance to extension for all three flours decreased with the addi-

tion of increasing quantities of thiosulfate. Sodium thiosulfate was more effective in small quantities, but less effective in large dosages, than sodium sulfite in reducing resistance to extension.

Largest extensogram areas were secured with thiosulfate when the dosage was 2.84 mg per 100 g of flour. Whether this quantity is the optimum would probably depend on the character of the flour.

Effect of Potassium Bromate and Sodium Sulfite Used Simultaneously. The compensating effect of a reducing agent on a dough treated at the

TABLE XI

EFFECT OF POTASSIUM BROMATE ON EXTENSOGRAM DIMENSIONS OF LEAVENED DOUGHS

Extensogram dimensions[1]	Yeast per 100 g flour	$KBrO_3$ per 100 g flour	Flours								
			Strong			Medium			Weak		
			Rest time, min			Rest time, min			Rest time, min		
			45	90	135	45	90	135	45	90	135
	g	*mg*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*
E_1	0	0	12.7	9.0	7.8	12.4	8.7	7.7	6.0	5.5	5.1
	3	0	10.3	8.0	7.6	10.0	8.3	6.8	5.6	4.2	3.6
	3	1	10.1	7.8	6.5	10.8	8.1	6.6	6.4	4.4	3.6
	3	2	10.9	8.3	7.3	10.4	6.5	6.0	5.9	4.4	3.8
	3	3	12.4	8.7	7.3	11.1	7.4	6.3	5.9	4.4	3.9
			eu	*eu*	*eu*	*eu*	*eu*	*eu*	*eu*	*eu*	*eu*
H	0	0	611	845	854	455	652	685	498	544	460
	3	0	397	423	405	240	302	290	261	267	238
	3	1	480	500	448	250	310	322	270	303	267
	3	2	508	508	475	307	335	367	303	330	290
	3	3	403	477	490	303	398	398	328	375	323
			cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2
A_1	0	0	86.0	79.0	66.4	65.3	60.6	53.2	32.8	30.4	24.4
	3	0	45.0	34.3	29.8	30.6	24.4	20.0	17.2	12.2	9.0
	3	1	51.2	37.9	30.3	33.0	25.0	19.8	20.1	13.7	9.5
	3	2	55.2	39.1	32.3	36.7	23.0	21.6	20.3	14.9	11.6
	3	3	54.7	40.1	32.0	39.8	33.2	23.7	21.2	16.8	12.3

[1] E_1 = Extensibility at the time of maximum resistance.
H = Maximum resistance to extension.
A_1 = Area enclosed by the curve and the base line at the time of maximum resistance.

same time with an oxidizing agent was studied, using potassium bromate and sodium sulfite as reagents. The dosages were varied to cover a considerable range in treatment. The data from this study are recorded in Table X. The effects of one reagent tended to be counteracted by the other, but the compensation was not entirely quantitative. The oxidation requirements of a flour appear to have an important bearing on the relative effect of the two reagents added simultaneously.

Effects of Oxidizing and Reducing Agents on Extensograms of Leav-

ened Doughs. The effect of varying dosages of potassium bromate on the extensograms of doughs containing 3% yeast (flour basis) is recorded in Table XI. The addition of 1 mg of potassium bromate had a tendency to decrease the extensibility as compared with the controls; except for the strong flour, greater additions tended to further decrease the extensibility, particularly with the medium strength flour. Resistance to extension and extensogram area increased as the quantity

TABLE XII

EFFECT OF SODIUM SULFITE ON EXTENSOGRAM DIMENSIONS OF LEAVENED DOUGHS

Extensogram dimensions[1]	Yeast per 100 g flour	$NaSO_3$ per 100 g flour	Flours								
			Strong			Medium			Weak		
			Rest time, min			Rest time, min			Rest time, min		
			45	90	135	45	90	135	45	90	135
	g	*mg*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*
E_1	0	0.00	12.7	9.0	7.8	12.4	8.7	7.7	6.0	5.5	5.1
	3	0.00	10.3	8.0	7.6	10.0	8.3	6.8	5.6	4.2	3.6
	3	2.27	13.0	9.4	8.9	10.5	8.0	6.8	6.1	4.6	4.2
	3	4.54	14.0	10.1	8.8	11.3	7.8	6.7	6.1	5.0	4.6
	3	6.81	12.0	9.9	8.7	10.9	9.7	8.3	6.8	5.9	5.6
			cu	*cu*	*cu*	*cu*	*cu*	*cu*	*cu*	*cu*	*cu*
H	0	0.00	611	845	854	455	652	685	498	544	460
	3	0.00	397	423	405	240	302	290	261	267	238
	3	2.27	377	403	417	333	435	422	338	366	345
	3	4.54	495	557	557	383	458	474	357	412	409
	3	6.81	443	523	590	188	255	300	168	222	245
			cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2
A_1	0	0.00	86.0	79.0	66.4	65.3	60.6	53.2	32.8	30.4	24.4
	3	0.00	45.0	34.3	29.8	30.6	24.4	20.0	17.2	12.2	9.0
	3	2.27	59.5	39.7	36.5	45.2	34.2	26.4	24.1	17.5	14.8
	3	4.54	74.1	56.6	46.4	52.0	36.6	33.1	25.6	21.7	19.6
	3	6.81	65.7	54.5	47.1	27.3	29.3	26.7	15.7	16.0	16.3

[1] E_1 = Extensibility at the time of maximum resistance.
H = Maximum resistance to extension.
A_1 = Area enclosed by the curve and the base line at the time of maximum resistance.

of bromate was increased. The areas, however, were not as great as when no yeast and no bromate were used in the doughs.

The effects of varying quantities of sodium sulfite on the extensograms of leavened doughs are recorded in Table XII. Extensibility of doughs prepared from all flours was increased when the sulfite was increased, and reached a maximum value with 4.54 mg per 100 g of flour. A further increase in the quantity of sulfite resulted in a marked decrease in resistance to extension. The areas of extensograms increased until 4.54 mg of sodium sulfite per 100 g of flour was used. At

that dosage the areas tended to be at a maximum value with all three types of flours.

Fats. Merritt, Blish, and Sandstedt (1932) found that shortening added to the baking formula increased loaf volume. Heald (1937) found it decreased gas production but improved gas retention and he recommended its inclusion in the baking test. Shellenberger (1941) visualized the shortening as coating starch and gluten surfaces with a

TABLE XIII

EFFECT OF FATS AND A MINERAL GREASE ON EXTENSOGRAM DIMENSIONS OF UNLEAVENED DOUGHS

Extensogram dimensions [1]	Fat added [2]	Dosage per 100 g flour	Flours								
			Strong			Medium			Weak		
			Rest time, min			Rest time, min			Rest time, min		
			45	90	135	45	90	135	45	90	135
		g	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*
E_1	—	0	12.7	9.0	7.8	12.4	8.7	7.7	6.0	5.5	5.1
	H.V.	3	12.8	8.6	7.3	12.4	9.2	7.9	6.3	5.6	5.1
	C.O.	3	9.8	7.6	6.8	13.5	9.9	8.3	7.5	6.0	5.6
	L.	3	12.1	7.9	7.3	13.8	10.5	9.0	7.2	6.0	5.3
	M.G.	3	11.7	9.1	7.8	12.8	9.8	8.2	7.1	5.9	5.3
			eu	*eu*	*eu*	*eu*	*eu*	*eu*	*eu*	*eu*	*eu*
H	—	0	611	845	854	455	652	685	498	544	460
	H.V.	3	627	889	851	458	655	682	475	552	463
	C.O.	3	708	865	767	440	614	665	500	561	571
	L.	3	601	787	758	434	633	643	482	597	527
	M.G.	3	628	855	867	416	595	684	407	493	483
			cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2
A_1	—	0	86.0	79.0	66.4	65.3	60.6	53.2	32.8	30.4	24.4
	H.V.	3	87.0	75.9	61.8	63.8	64.6	59.1	32.5	31.4	24.2
	C.O.	3	73.1	65.2	48.3	67.3	65.2	56.1	41.6	34.3	32.2
	L.	3	80.5	66.3	57.3	68.7	72.1	59.9	39.3	37.7	30.7
	M.G.	3	81.0	80.4	68.3	62.7	63.8	59.0	32.8	30.4	26.3

[1] E_1 = Extensibility at the time of maximum resistance.
H = Maximum resistance to extension.
A_1 = Area enclosed by the curve and the base line at the time of maximum resistance.
[2] H.V. = Hydrogenated vegetable oil.
C.O. = Cottonseed oil.
L. = Lard.
M.G. = Mineral grease.

thin film which acted as an internal lubricant. Baker and Mize (1939, 1942) have also studied the effects of fats in breadmaking. They observed that semisolid fats improved bread quality, whereas the liquid fats had little effect. They considered the improving action of fats in baking to be due to their reducing the size of, or plugging, the pores through which gas escaped during baking, and that only the solid or semisolid fats act in this manner.

The effect of three types of shortening and a mineral grease was investigated and the data are recorded in Table XIII. The shortening fats were a hydrogenated vegetable shortening, cottonseed oil, and leaf lard. The mineral grease was of the type usually called cup grease.

The data indicate that fats differ in their effects on doughs made from flours of different strength. Extensibility was decreased when fats were added to strong doughs but was increased when they were added to doughs of the weaker flours. Hydrogenated vegetable shortening and cottonseed oil increased the resistance to extension of

TABLE XIV

EFFECT OF NONFAT MILK SOLIDS ON EXTENSOGRAM DIMENSIONS OF UNLEAVENED DOUGHS

Extensogram dimensions[1]	Nonfat milk solids per 100 g flour	Flours								
		Strong			Medium			Weak		
		Rest time, min			Rest time, min			Rest time, min		
		45	90	135	45	90	135	45	90	135
	g	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*
E_1	0	12.7	9.0	7.8	12.4	8.7	7.7	6.0	5.5	5.1
	3	12.3	8.7	7.8	12.2	9.2	8.1	7.7	5.8	5.4
	6	11.9	9.1	7.8	11.1	8.7	7.1	6.2	5.3	5.1
		eu	*eu*	*eu*	*eu*	*eu*	*eu*	*eu*	*eu*	*eu*
H	0	611	845	854	455	652	685	498	544	460
	3	641	900	922	434	664	710	427	516	524
	6	591	912	941	419	600	633	388	472	423
		cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2	cm^2
A_1	0	86.0	79.0	66.4	65.3	60.6	53.2	32.8	30.4	24.4
	3	89.0	80.6	69.6	62.7	65.1	64.7	36.8	31.0	29.7
	6	81.8	85.1	72.7	54.7	55.8	46.0	27.4	27.1	23.8

[1] E_1 = Extensibility at the time of maximum resistance.
H = Maximum resistance to extension.
A_1 = Area enclosed by the curve and the base line at the time of maximum resistance.

the strong doughs. The shortening had little effect on the resistance to extension of the medium strength doughs; with the weak doughs cottonseed oil and lard increased the resistance to extension. The effect of fats on area of extensograms was variable; in the strong doughs they tended to decrease extensogram area, whereas in medium-strength and weak doughs they tended slightly to increase the area.

Nonfat Milk Solids. The effect of nonfat milk solids on the physical properties of unleavened doughs was studied at two levels, 3% and 6%. Extra water was added so that the consistency of the doughs was kept reasonably constant. The data recorded in Table XIV show that

nonfat milk solids had no very pronounced nor consistent effect on the properties of doughs as measured with the extensograph.

Extensogram Properties of Fermented Doughs at the End of the Proof Period

In the test baking of wheat varieties at the Minnesota Agricultural Experiment Station, it has been customary to use the A.A.C.C. baking formula (with the addition of potassium bromate where necessary to secure satisfactory development) and to employ two mixing periods (2 and 4 minutes) and two fermentation times (2 and 3 hours). By this means four different treatments with a fixed formula are applied

TABLE XV

LOAF VOLUMES AND EXTENSOGRAM DIMENSIONS OF FERMENTED DOUGHS AT BAKING TIME

Flour	Fermentation period	Mixing period	Loaf volume	Extensogram dimensions [1]		
				E_1	H	A_1
	hr	*min*	*cc*	*cm*	*eu*	*cm²*
Strong	2	2	758	6.7	413	31.8
	3	2	760	6.8	370	28.4
	2	4	848	5.8	402	28.1
	3	4	750	5.4	357	23.8
Medium	2	2	698	6.4	364	25.5
	3	2	660	6.4	304	21.2
	2	4	758	5.6	354	21.8
	3	4	682	5.6	288	19.2
Weak	2	1	455	5.8	272	15.7
	3	1	465	5.0	230	12.5
	2	2	512	5.4	322	17.8
	3	2	488	5.3	295	17.4

[1] E_1 = Extensibility at the time of maximum resistance.
H = Maximum resistance to extension.
A_1 = Area enclosed by the curve and the base line at the time of maximum resistance.

and a considerable range in the severity of the test is obtained. It was of interest to determine the physical properties of doughs, prepared according to the procedures just described, at the end of the regular proof period of 55 minutes. Each dough was mixed in a Hobart-Swanson mixer and, immediately after mixing, was scaled into two 150-g aliquots. Both were handled as for baking except that one of the two, at panning time, was formed into a test piece in the extensograph, allowed to rest for 55 minutes, and then extended as in the usual testing procedure. The other dough aliquot was panned, proofed, and baked in the normal way. The average data from duplicated tests are shown in Table XV.

With the exception of the strong flour at 3 hours fermentation, the

loaf volumes increased with an increase in mechanical development at mixing time, especially at the shorter fermentation period. Extensibility tended to decrease with the longer mixing treatment. Resistance to extension decreased when the fermentation period was lengthened; it also decreased slightly with increased mixing, except with the weak flour. With this flour, resistance increased with both lengthened mixing and fermentation times, although the mixing periods involved in these comparisons were only half as long as with the strong and medium-strong flour doughs. With the strong and medium-strong flours, extensogram areas decreased as both fermentation and mixing periods were lengthened. When the weak flour was used, the extensogram area decreased with lengthened fermentation but increased when the doughs were given more mechanical development.

Prediction of Bromate Requirements of Doughs

For the production of good bread, it is essential to have the dough at optimum extensibility. The optimum value varies for different flours; also the extensibility of doughs made from different flours is not affected uniformly by the addition of various ingredients or by fermentation. The changes in extensibility, with time, or with some other treatment producing similar results, are accompanied by a decrease in resistance to extension. As pointed out by Munz and Brabender (1940), the ratio of resistance to extensibility does not serve to distinguish between flours exhibiting considerable differences in other properties. Neither does the change in this ratio with time or oxidizing treatment, alone, denote the strength of a flour. Munz and Brabender (1940) used the ratio F/E to characterize the extensogram, where F was the force in extensogram units required to extend the dough and E was the total extension in millimeters. However, they used the total extension, which, in this investigation, has been too variable to be reliable. They also evolved a formula that included the area, A, enclosed by the base line and the extensogram curve, together with the F/E ratio in an expression, $\frac{A}{F/E \times 10}$, as a measure of the oxidation requirements supplied by potassium bromate. They called this number the oxy-number.

Areas of the extensograms, or portions of them, might be expected to be an important index of some characteristic of a flour. In one sense it is, for largest areas were secured with the strongest flour which in turn could be baked into the largest loaves. In the present study, values for the expression $\frac{A_1}{H/E}$ or $\frac{A_1E}{H}$ were computed. They generally decreased with time and with increased severity of oxidizing treat-

ments, whereas they generally increased with an increase in severity of reducing treatments. However, the values for doughs made from strong and weak flours were not correlated with the baking results. Apparently some important factor was omitted. Accordingly, the protein content, P (15% moisture basis), was brought into the expression and the value $\frac{0.1\,PH}{A_1E_1}$ was computed from the data recorded in Tables IV and VII for doughs made with the strong, medium, and weak flours prepared with water and salt alone as well as with three

TABLE XVI

EFFECTS OF INCREMENTS OF POTASSIUM BROMATE AND OF SODIUM SULFITE ON COMPUTED VALUES OF $\frac{0.1\,PH}{A_1E_1}$

Reagent	Dosage per 100 g flour	Flours								
		Strong			Medium			Weak		
		Rest time, min			Rest time, min			Rest time, min		
		45	90	135	45	90	135	45	90	135
	mg									
$KBrO_3$	0	0.65	1.56	2.13	0.55	1.04	1.43	1.42	2.27	2.68
	1	1.04	2.37	3.42	0.54	1.27	1.50	1.45	2.56	2.97
	2	1.27	2.90	3.59	0.62	1.44	1.96	1.54	2.91	3.26
	3	1.10	2.53	3.72	0.70	1.49	2.16	1.88	3.13	3.96
Na_2SO_3	0.00	0.65	1.56	2.13	0.55	1.04	1.43	1.42	2.27	2.68
	2.27	0.64	1.38	1.70	0.51	0.93	1.49	1.54	2.70	2.92
	4.54	0.56	1.34	1.71	0.47	0.82	1.07	1.11	2.11	2.72
	6.81	0.60	1.27	1.70	0.40	0.65	0.82	0.65	1.72	2.03

increments each of potassium bromate and of sodium sulfite at three different rest periods. The results are recorded in Table XVI.

Values for $\frac{0.1\,PH}{A_1E_1}$, which might be called the "age-index," increase with rest time and with the dosage of potassium bromate; in general, the larger quantities of sodium sulfite tended to give lower values of the "age-index." The "age-index" values should not be expected exactly to parallel loaf volumes, Tables V and VIII, even within groups of flours of the same type, because other factors than those encountered in unleavened doughs tested with the extensograph affect loaf volume.

The "age-index" value is proposed as an indication of the condition of the dough at the time it is tested and, knowing the time and other conditions imposed with the test, the future conduct of the dough may

be predicted. On comparing the data in Table XVI with those in Tables V and VIII for the strong flour, values from 0 to 1.0 indicate "young" doughs that are capable of considerable response to oxidizing treatment or to longer fermentation. Values from 1.0 to 1.5 indicate mature doughs that will show but little response to more fermentation or oxidation. Doughs having values from 1.5 to 2.5 are slightly overmature but will not suffer much reduction in loaf volume due to the overtreatment. Doughs giving rise to values over 2.5 are already overdeveloped, and further oxidizing treatment will tend to result in a definite negative response in loaf volume.

The doughs prepared from the medium-strength flour used in these tests gave slightly lower "age-index" values than those for the strong flour.

It is recognized that the utility of "age-index" is limited both by the number of factors acting simultaneously on a dough and by the ability of the operator to interpret the data. Past experience and the results of the present investigation point to the necessity for extreme care in conducting and interpreting physical measurements of doughs. The very sensitivity of the extensograph makes good replication of results impossible without avoiding any deviations from a standard procedure.

Summary

Replicability of extensograph tests was good when a standard procedure involving the use of unleavened doughs was employed. Tests repeated on the same dough rather than single tests after varying rest periods are recommended.

Oxidizing action of potassium bromate and potassium iodate effected a decrease in extensibility and in the area enclosed by the extensogram, and an increase in resistance to extension. Two and three successive testings of doughs treated with potassium bromate effected greater reduction in area of extensograms than did the corresponding tests of doughs containing potassium iodate. A positive loaf volume response to potassium bromate was obtained with a strong and medium-strength flour but not with a weak flour.

Reducing agents such as sodium sulfite and sodium thiosulfate effected an increase in extensibility and a decrease in resistance to extension of doughs. Extensogram area was increased slightly by moderate dosages of sodium sulfite and decreased by sodium thiosulfate. Reducing action of sodium sulfite had little effect on the loaf volumes of a strong and a medium-strength flour but increased the loaf volumes of a weak flour.

Simultaneous addition of potassium bromate and sodium sulfite to doughs resulted in a compensatory effect. The compensating action of these reagents was not quantitative but varied with the quantities used and with different flours.

Leavening action of yeast decreased all dimensions of extensograms but was partially counteracted by the addition of either potassium bromate or sodium sulfite to the formula.

Shortenings of several types behaved differently with different flours. In general, they increased extensibility but had no uniform effect on resistance or extensogram area.

Nonfat milk solids decreased extensibility and resistance to extension with flours of low protein content. A dosage of 3 g of nonfat milk solids per 100 g of flour increased extensogram area and stabilized doughs against the effect of repeated tests to a greater extent than a dosage of 6 g per 100 g of flour.

Prolonged mixing decreased the extensibility and extensogram areas of fermented doughs at baking time and tended to decrease resistance to extension. Lengthened fermentation decreased resistance and extensogram areas when measured at the end of the proof period.

"Age-index" values of doughs may be computed from a formula involving the protein content of the flour and measurements characterizing the extensogram. These values may prove useful in predicting the bromate requirements of doughs.

Literature Cited

Bailey, C. H.
1940 Physical tests of flour quality. Wheat studies of the Food Research Institute 16: 243–300.

Baker, J. C., and Mize, M. D.
1939 Effect of temperature on dough properties. II. Cereal Chem. 16: 682–695.
1942 The relation of fats to texture, crumb, and volume of bread. Cereal Chem. 19: 84–94.

Blish, M. J.
1934 Treatment of grain products. U. S. Patent No. 1,957,688.

Heald, W. L.
1937 The effect of different types of shortening on white pan bread. Cereal Chem. 14: 481–488.

Merritt, P. P., Blish, M. J., and Sandstedt, R. M.
1932 Report of activities of A.A.C.C. baking research fellowship. Cereal Chem. 9: 175–238.

Munz, E., and Brabender, C. W.
1940 Extensograms as a basis of predicting baking quality and reaction to oxidizing agents. Cereal Chem. 17: 313–332.

Shellenberger, J. A.
1941 The effect of shortening on the A.A.C.C. bread-baking test. Cereal Chem. 18: 224–231.

Stamberg, O. E., and Merritt, P. P.
1941 Quantity of dough in relation to the use of the farinograph. Cereal Chem. 18: 627–632.

Swanson, C. O.
1943 Physical properties of doughs. Burgess Publishing Co. Minneapolis, Minnesota.

MIXING TOLERANCES OF VARIETIES OF HARD RED SPRING WHEAT [1]

T. R. AITKEN and M. H. FISHER

Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba

(Received for publication May 26, 1945)

Mixing tolerance is an important and widely studied property of flour. Aside from such observations as can be made during the mixing of the dough, methods for measuring this property include the use of recording mixers, notably the farinograph and the mixograph, and baking tests in which mixing time is varied. A standardized form of the latter procedure, particularly suitable for comparing new varieties of hard red spring wheat, has been adopted in this laboratory. Single loaves are baked from seven doughs representing a series of mixing times, loaf volumes are plotted against time, and a smooth curve is fitted to the points. These curves can be readily compared, their interpretation is simple and reasonably objective, and they appear to offer more comprehensive and convincing evidence of differences between varieties than is given by most other tests.

Results obtained with this procedure for a number of varieties are given in this paper, and comparisons are made with mixograms and farinograms.

Materials and Methods

The principal studies were made with varieties grown in replicate rod-row plots in 1943 and 1944 at a number of stations in western Canada. There were 15 varieties from nine stations in 1943, and 16 varieties from 12 stations in 1944. In each year a composite sample was made up for each variety by taking equal parts of grain from each station.

A study of the effects of protein content on mixing characteristics was also made with 20 samples of Thatcher wheat grown in 1943. These were selected to cover a range of protein content from a large number of samples that had been obtained from registered seed growers farming in western Canada.

For the 1943 studies, duplicate loaves were baked from doughs mixed for 1, 2, and 3 minutes in the Hobart-Swanson mixer. The malt-phosphate-bromate formula was used (Geddes, Aitken, and Fisher, 1940). In the following year a better procedure was devised;

[1] Paper No. 78 of the Grain Research Laboratory and Paper No. 237 of the Associate Committee on Grain Research (Canada). Presented by J. A. Anderson, F.R.S.C., to the Royal Society of Canada, May, 1945.

single loaves were baked with doughs mixed for 1, 1.5, 2, 2.5, 3, 3.5, and 4 minutes. The time at which definite stickiness of the dough occurred was also noted. Although the loaves were scored for the usual properties, these data are not recorded because they tend to follow loaf volumes, and because these properties are of secondary importance in this type of study. In the second series, loaf volume was plotted against mixing time, and curves were fitted by statistical methods.

Farinograms were made with a 50-g mixer by the method given by Geddes *et al* (1940). Mixograms were made with 35 g of flour (14% moisture basis), with the spring set in notch "eleven," and at the absorption used for baking.

Effect of Protein Content on Curves for Loaf Volume and Mixing Time

The curves for loaf volume on mixing time for the 20 samples of Thatcher are shown in the left half of Figure 1. They are arranged in decreasing order of initial loaf volume. As would be expected when dealing with samples from individual fields, loaf volumes at each mixing time do not fall strictly in the order of protein content, although the number of exceptions is small, particularly with the shortest mixing time. For most of the samples, loaf volume has a maximum value for two minutes mixing and the value for three minutes mixing is about equal to that for one minute mixing. There is definite evidence that the increase in loaf volume from one to two minutes mixing decreases with decreasing protein content. This can be illustrated by plotting curves for each group of four samples counting from the highest protein level. This has the effect of averaging out differences among individual samples and brings to light the principal trend. The curves for the five groups of samples are shown in the right half of Figure 1. They show that as protein content decreases, the over-all level of the curve falls and it also tends to flatten. In short, at higher protein content the increase in loaf volume that can be obtained with additional mixing is greater and the flour may thus be said to have more reserve strength. Although the drop in loaf volume from two to three minutes mixing is greater for the higher protein flours, this does not necessarily mean that the lower protein flours can withstand overmixing better. As pointed out by Aitken and Geddes (1934), such a conclusion would be fallacious because under optimum conditions low protein flours give loaves that are close to the border line of what may be considered as satisfactory, and such flours are insensitive to further changes resulting from adverse baking conditions. On the other hand, high protein flours yield loaves of large volume under optimum conditions and, because of their sensitivity to changes brought about by severe conditions, they can suffer a

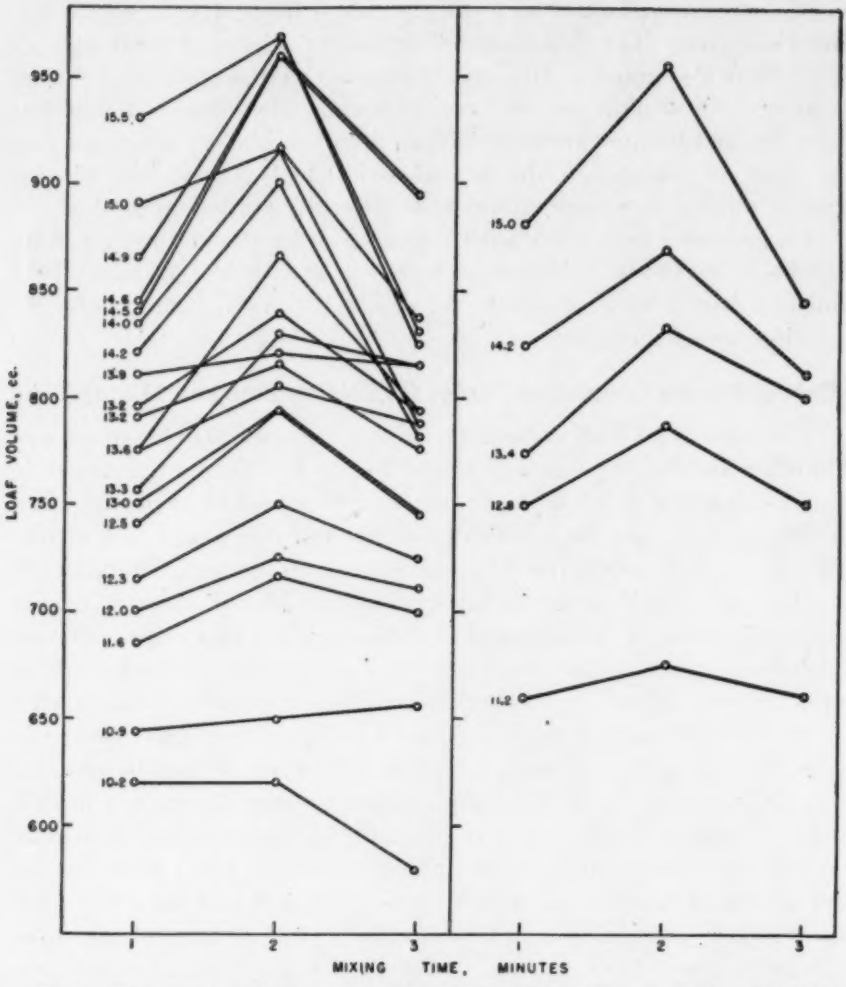


Fig. 1. Curves for loaf volume on mixing time for 20 samples of Thatcher wheat grown in 1943. The curves on the left are for individual samples, and the curves on the right are for each group of four samples counting from the top. The figures at the origins of the curves represent the values for protein content.

greater reduction in loaf volume and still yield loaves of much higher volume than low protein flours when baked under corresponding conditions.

Characteristics of Varieties

Similarity between Years. Curves for loaf volume on mixing time for corresponding varieties grown in 1943 and 1944 are shown in Figure 2.

In comparing the corresponding curves for the two years, due allowance must be made for differences in protein content for, as indi-

cated in the previous section, the level of a curve is a reflection of the protein content of the flour. In most samples, the level of the curve is higher for the 1943 samples and, with one exception, protein content is also higher. Bearing this in mind it is apparent that agreement on curve type between corresponding varieties is remarkably good. No. 10 is the only variety whose curve type is quite different in the two years; in 1943 it resembled the Thatcher curve and in 1944 it resembled the Marquis curve. There is thus definite evidence that the curve for loaf volume on mixing time is a varietal characteristic when comparisons are restricted to varieties with a narrow spread in protein content. In 1943, the range in protein content of the 15 varieties was 2.6% with 11 falling within the range 13.0 to 14.4%; and in 1944 the range in protein content for the 16 varieties was 2.7% with 11 falling within the range 12.8 to 14.1%.

1944 Curves for Loaf Volume on Mixing Time. Intervarietal differences are discussed for the 1944 samples represented by the smooth curves in Figure 2. Although varietal differences were indicated quite definitely by the three-point curves, the use of only three mixing times made it impossible to determine whether the largest loaf volume obtained was the potential maximum for a variety; it might have been obtained with a slightly shorter or a slightly longer mixing time. Employment of a larger number of mixing times with shorter intervals between them eliminates this possibility, and it is also believed that a smooth curve fitted to seven points is more informative than a sharp-peaked curve joining only three points. Although seven loaves are required for the smooth curves, this entails only one extra loaf, because the values for the three-point curves were means for duplicate loaves and single loaves are adequate for the seven-point curves.

In evaluating the smooth curves the following points are considered: (1) over-all shape, which, when compared with that of an accepted standard variety, types a variety for its loaf volume responses to mechanical dough development; (2) mixing time over which initial loaf volume is maintained, which is a measure of mixing requirements and mixing tolerance; (3) difference in loaf volume between the initial and maximum values, which is a measure of extra or reserve strength; (4) time when stickiness in the dough first occurs, which is indicated on the abscissa by an arrow; and (5) general level of the curve, which is an index of over-all baking strength. Whenever a curve is definitely lower than that of the standard variety, it is differentiated for its low baking strength regardless of its over-all shape. Such a curve might be the same or even more desirable (flatter) than that of the standard variety, but it would represent a relatively weak flour that is not sensitive to changes in baking procedure.

Reading from left to right in Figure 2 an attempt has been made to arrange the 16 varieties to show a trend in decreasing over-all strength as indicated by the curve characteristics. Protein data for the 16 varieties are given below:

Variety No.	1	2	3	4
Protein, %	13.6	14.1	13.0	14.6
Variety No.	5	6	7	8
Protein, %	13.9	13.6	14.5	13.5
Variety No.	9	10	11	12
Protein, %	13.2	13.3	14.5	13.8
Variety No.	13	14	15	16
Protein, %	13.1	12.8	13.3	11.9

As protein content affects the position and shape of the curve, it is apparent that the order of the variety curves in Figure 2 is partly the result of differences in protein content. But there is no consistent relation between over-all strength, as indicated by the curves, and protein content. Moreover, since all the varieties were grown side by side, differences in protein level and such effects as these have on the curves are directly attributable to differences in the varieties themselves.

Among the varieties represented in Figure 2 there are several distinct groups within which similar baking properties are indicated. The first group includes Nos. 1, 2, and 3. The over-all levels of the curves are quite high and they decrease progressively from No. 1 to No. 3. This indicates that within this group No. 1 is the strongest flour, No. 3 the weakest, and No. 2 intermediate. Initial loaf volumes were maintained for almost the full mixing time, and stickiness in the dough did not occur until about three minutes or longer. These three varieties would therefore be expected to give a good performance with both a short and a long mixing time. The differences between initial and maximum loaf volumes also indicate satisfactory levels of extra strength. The three varieties are definitely stronger than Marquis (No. 13).

The next group includes No. 4, Thatcher, No. 6, and Regent, and like the first group the levels of the curves decrease, thus indicating decreasing over-all strength. Initial strength for No. 4, Thatcher and No. 6, and Regent is, respectively, superior to that of No. 1, No. 2, and No. 3, but the four curves show a marked falling-off in loaf volumes after about three minutes mixing time. On the whole, stickiness in the dough also occurred somewhat earlier in this second group of samples. Extra strength is quite satisfactory, but it decreases from No. 4 to Regent. No. 4 and Thatcher would be expected to do well with both

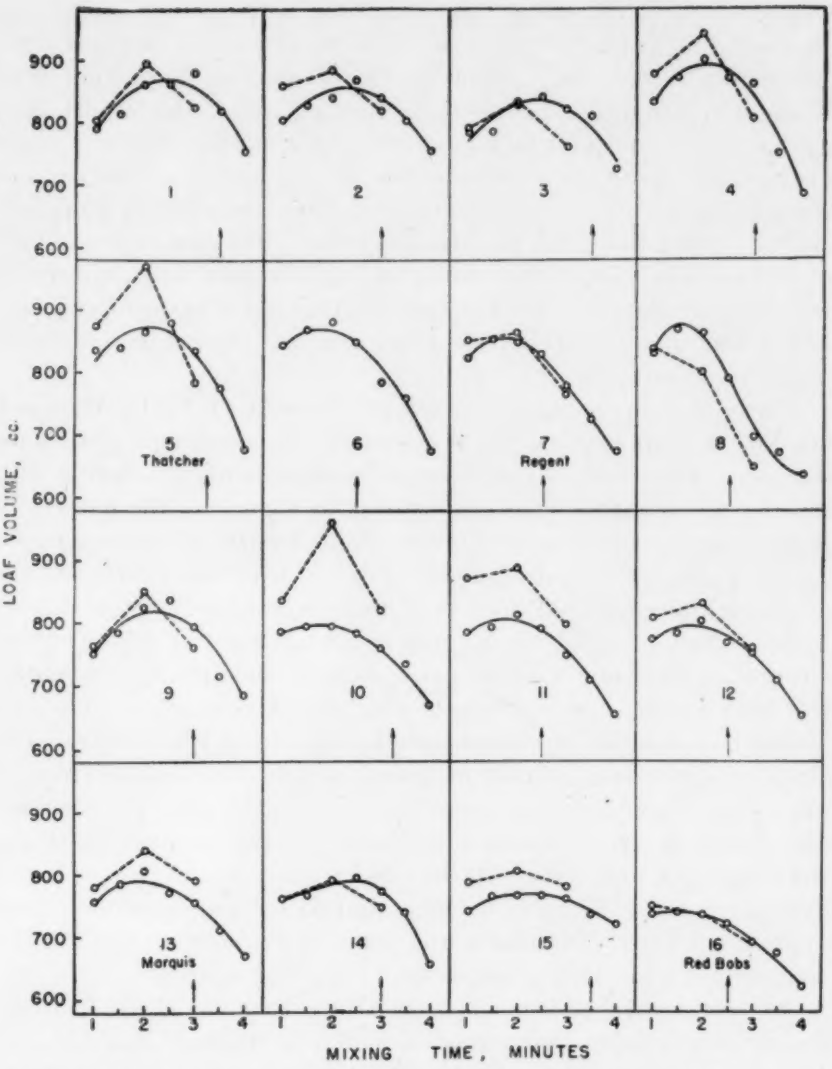


Fig. 2. Curves for loaf volume on mixing time for different varieties of wheat grown in 1943 (broken lines) and 1944 (solid lines). The arrows indicate the times when the doughs became sticky.

a short and a relatively long mixing time, but No. 6 and Regent would not withstand prolonged mixing to the same extent—the curves are flatter and stickiness of the dough occurs earlier. The four varieties in this group are classed as stronger than Marquis.

The curve for No. 8 is in a class by itself. Although initial strength of this variety is high, it is maintained for only a relatively short mixing period and after this point the curve falls off rapidly to about the lowest level of all the varieties. Good extra strength is also indicated

over a narrow mixing range, and stickiness in the dough occurred at the same time as that for Regent. This variety would be suitable for short mixing times only. Although this variety would be classed as strong, it is extremely sensitive to overmixing, and in this it falls short of what is expected in a high-grade western Canadian wheat. No. 9 gives a curve that is about intermediate in type between Thatcher and Marquis; but its over-all level is distinctly lower than that of Thatcher, and the curve falls off like the Marquis curve. Had the degree of falling off been less it would have followed No. 3, because in some respects the curves are similar. No. 9 is a variety that would perform well with both a long and a short mixing time, and it is classed as a stronger sample than Marquis.

The next group includes five varieties (Nos. 10, 11, 12, 13 [Marquis], and No. 14) that have curves of essentially the same type as that for Marquis. The curve levels indicate medium-strong varieties with some extra strength. Mixing tolerance, as shown by the flatness of the fore part of the curves, is quite good. Dough stickiness covered a narrow range, 2.5 to 3 minutes. The chief difference between the samples in this group and those in the second group is the over-all level of the curves; it is higher for the second group but otherwise the patterns are similar. This group of samples would prove satisfactory with both a short and a relatively long mixing time.

The last group includes two varieties, No. 15 and Red Bobs. The curves indicate wheats of only medium strength whose baking strength is sufficiently low under optimum baking conditions as to make them insensitive to severe baking conditions. Extra strength is slight, particularly for Red Bobs. Of the two varieties, No. 15 has the better over-all strength; the curve is higher and flatter and dough stickiness occurs much later. Neither variety, however, possesses the kind of strength desired in high-grade western Canadian wheat.

As indicated by the curves, three of the varieties (Nos. 4, 6, and Regent) have mixing characteristics similar to those of Thatcher, and four of the varieties (Nos. 10, 11, 12, and 14) have mixing characteristics similar to those of Marquis. Nos. 1, 2, 3, and possibly 9 are superior to Thatcher in mixing tolerance, and they fall somewhere between Thatcher and Marquis in over-all strength. The curves for Nos. 1, 2, and 3 indicate very strong varieties that are distinctly superior to Marquis. No. 8 would appeal to purchasers who prefer a strong wheat that performs well with a short mixing time, but it is particularly sensitive to mixing and has a narrow range in mixing tolerance. No. 15 and Red Bobs, particularly the latter, lack the kind of strength desired in high-grade western Canadian wheat.

1944 Mixograms. The mixograms for the 16 varieties are reproduced in Figure 3. They are arranged in the same variety order as the curves for loaf volume on mixing time (Figure 2). According to Larmour, Working, and Ofelt (1939), mixograms show the mixing behavior of varieties by the rate at which the dough can be mixed to maximum consistency and the manner in which it behaves after this maximum has been passed. Swanson and Johnson (1943) state that the range in mixing tolerance of a flour is shown on the mixogram by the angle formed between the developing line (ascending slope) and the weakening line (descending slope). In a later paper (Johnson, Swanson, and Bayfield, 1943), these authors apply the term "range-of-

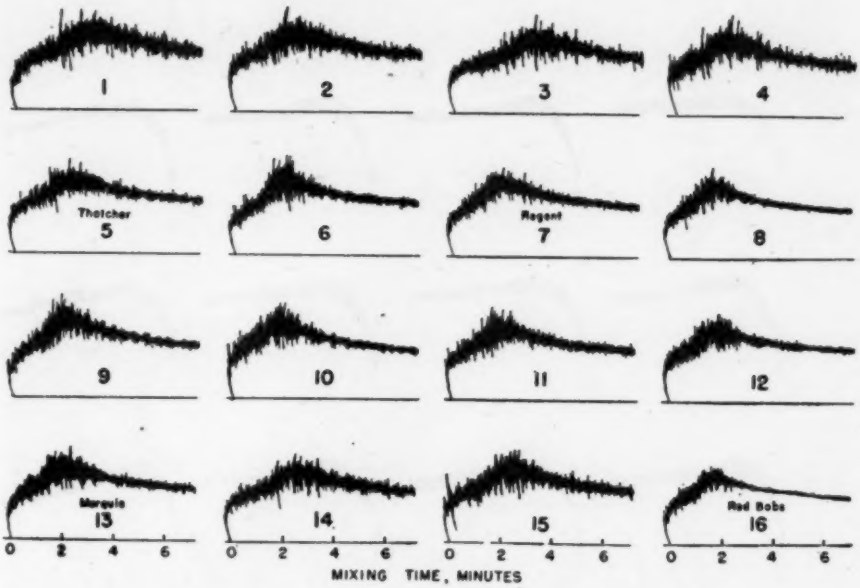


Fig. 3. Mixograms for different varieties of the 1944 crop.

tolerance angle" to this measurement. This dimension was therefore used in evaluating the curves for mixing tolerance. In accordance with general practice, the width of the mixogram and its pattern were used in evaluating the strength properties of the varieties.

The mixograms show quite wide variations in curve pattern of the 16 varieties, but they do not show the same trend in decreasing over-all strength as do the curves for loaf volume on mixing time. In general, the top row of curves indicates very strong flours with a wide range in mixing tolerance, and the second, third, and fourth rows include curves that indicate decreasing strength and narrowing mixing tolerance. However, there are a number of varieties that would be placed differ-

ently to be consistent with the arrangement of the curves for loaf volume on mixing time. The mixograms for Nos. 14 and 15 are doubtless misplaced; the former is closely similar to curve No. 2 and the latter is almost identical to curve No. 4. Both curves indicate high strength and a wide range in mixing tolerance. Curve No. 8 certainly fails to indicate the unusual mixing characteristics of this variety, and in pattern it is not unlike the Marquis curve. The curves for loaf volume on mixing time placed No. 14 in the same class with Marquis,

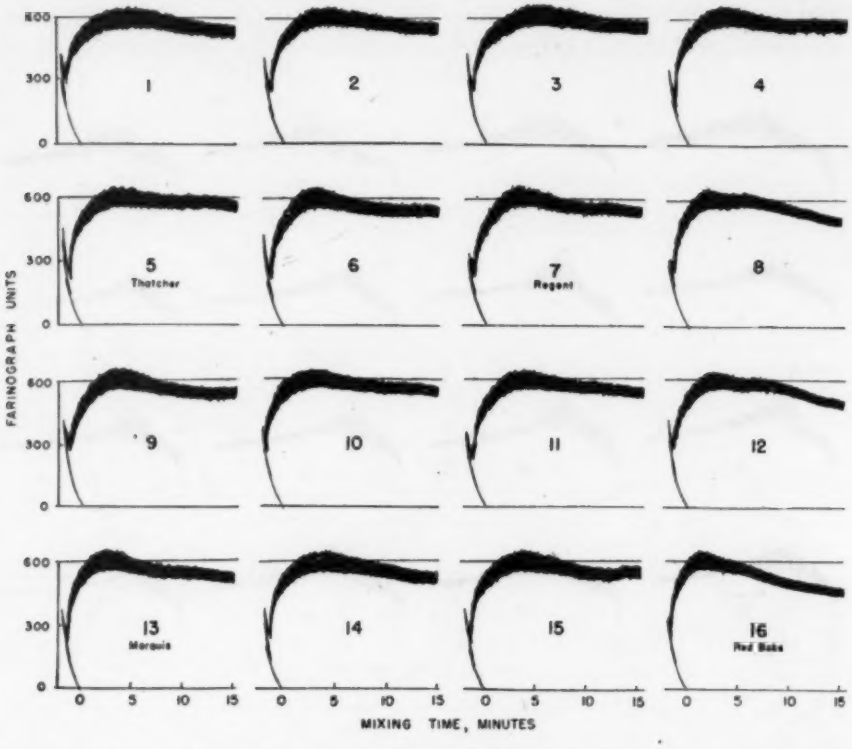


Fig. 4. Farinograms for different varieties of the 1944 crop.

No. 15 was faulted for its general low strength, and No. 8 was separate and distinct from any other variety. The mixogram for No. 3 would doubtless place it ahead of No. 2; its strength is about equal to No. 2 but its mixing tolerance is wider. On the other hand, the mixogram for No. 4, and possibly for No. 11, is a Thatcher type curve; mixograms for Regent, Nos. 8, 10, 11, and 12 are Marquis type curves.

1944 Farinograms. The farinograms for the 16 varieties are reproduced in Figure 4, and like the mixograms they are arranged in the same variety order as the curves for loaf volume on mixing time. The

strength properties of the farinograms were evaluated by the development time, band width, and curve pattern. While this is the customary interpretation of farinograms, it has previously been pointed out by Geddes, Aitken, and Fisher (1940) that these measurements are not always reliable indices of baking strength, and the results of the present study support this conclusion. The recommendations of Munz and Brabender (1940) were followed in interpreting the curves for mixing tolerance. These authors state that the farinogram indicates mixing tolerance by the slope of the curve after it reaches the point of maximum consistency, taking into account the development time. They believe that when two flours give curves with the same downslope but different development times, the one with the shorter development time is more sensitive to mixing because the angle between the ascending and descending slopes of the curve is smaller. It is thus apparent that farinograms and mixograms are interpreted in much the same way to indicate mixing tolerance..

The range in farinogram type is fully as wide as that for mixogram type, but like the mixograms they do not show the same trend in decreasing over-all strength as do the curves for loaf volume on mixing time. Moreover, the placings by the farinograms do not always agree with the placings by the mixograms. For example, by the farinograms No. 3 would definitely rank first, No. 2 is superior to No. 14, No. 6 and Regent are essentially the same, and Marquis is distinctly superior to No. 12. By the mixograms No. 3 is not superior to No. 1, No. 14 is superior to No. 2, Nos. 6 and 7 are quite different, and No. 12 is not materially different from Marquis. The farinogram for No. 8, however, shows the unsatisfactory mixing characteristics of this variety much better than the mixogram, but it fails to place the sample apart from the others as does the curve for loaf volume on mixing time. The farinograms confirm the mixograms for Nos. 14 and 15 and for Nos. 12 and 8; the former have curve patterns that indicate quite strong flours, and the latter have patterns that would place them respectively next to Red Bobs which is rightly placed last. The farinograms classify Nos. 2, 4, 10, and possibly 11 as Thatcher types; and Nos. 6, 9, and Regent as Marquis types.

Opinions of Other Chemists on the Quality of Four of the 1944 Varieties

Six of the varieties grown in rod-row plots in 1944 were also grown in one-tenth acre plots for a large collaborative study. The varieties were Nos. 4, 8, 12, 14, Marquis, and Thatcher, and each was a composite sample made up from equal parts of the variety from each station. The named varieties were included as standards for comparison.

Nineteen chemists in Great Britain, the United States, and Canada participated in the study. Each collaborator was asked to make the tests using whatever methods he preferred and to supply information on the principal differences between each unnamed variety and the two standard varieties, and the order of placing the six varieties for general over-all quality. The tests made by the Grain Research Laboratory included curves for loaf volume on mixing time, and it is of interest to compare our opinions on the strength and mixing characteristics of the samples with those of other collaborators who used different testing procedures. As the curves for the one-tenth acre varieties are almost identical in type with those for the corresponding rod-row varieties (Figure 2), they are not reproduced.

Variety No. 4. This variety ranked second, between Thatcher and Marquis, by the mean placing of all collaborators for all-round quality. Only three chemists classed it as inferior to Marquis; two because of the "bucky" character of the dough, and one because its strength was somewhat lower. The consensus of opinion was that No. 4 was high in strength and quite satisfactory for domestic and overseas use.

The curve for loaf volume on mixing time showed that No. 4 corresponded so closely to Thatcher in over-all strength, mixing requirements, and mixing tolerance as to be barely distinguishable from it. It was bracketed with Thatcher in first place and was classed as superior to Marquis. No. 4 was classed as a wheat that should appeal to millers who desire a high protein wheat of the Thatcher type, and in this there is good agreement with the majority of the other collaborators.

Variety No. 8. This variety ranked fourth by the mean placing for all collaborators and was classed as inferior to both Thatcher and Marquis. Twelve chemists placed it fourth or lower and six placed it last; but five chemists placed it either first or second. Several chemists faulted it for its unsatisfactory mixing and fermentation tolerance and one chemist favored it because it possessed shorter than normal mixing requirements. Strength properties were considered satisfactory by several chemists. Softening of the dough during fermentation and unsatisfactory tolerance to mixing and fermentation appeared to be its chief faults.

The curve for loaf volume on mixing time placed this variety apart from the others as the poorest in mixing tolerance. It had good initial strength, but the level was maintained for only a short mixing period. It is particularly sensitive to mixing, and for this reason was considered inferior to both Thatcher and Marquis and placed last. This variety was classed as one that would appeal to millers who favor a high protein wheat that bakes well with a short mixing time but not with a long

mixing time. Its inferiority to the two standard varieties was confirmed by many collaborators and several agreed with the unsatisfactory mixing characteristics shown by the curve.

Variety No. 12. This variety ranked fifth, slightly below No. 8, by the mean placing for all collaborators, and below both Thatcher and Marquis. Very few chemists included it in the higher bracket for all-round quality and the majority placed it fifth or sixth. Lack of strength appeared to be its chief fault and some chemists classed it as a soft wheat type because of its dough and bread making properties. Its low placing was doubtless a reflection of its unsatisfactory over-all strength in which it differed markedly from high-grade western Canadian wheat.

The curve for loaf volume on mixing time was flatter and slightly below that for Marquis, but the variety was not sensitive to mixing. It had little extra strength and mixing tolerance was nearly but not quite equal to that of Marquis. No. 12 was classed as not quite equal to Marquis in general quality and definitely inferior to Thatcher. It was placed about on a par with Marquis in mixing characteristics and in general strength. It was classed as a variety that would appeal to millers who desire a high protein medium-strength wheat that gives its best performance with a short mixing time. All things considered, its evaluation by the curve for loaf volume on mixing time was in good agreement with that by the other collaborators.

Variety No. 14. This variety was rated the poorest of the six varieties by the mean placing for all collaborators. Only three chemists placed it equal or superior to Marquis. Below average protein content was the chief reason for its low rating, but several of the collaborators noted its good response to mixing and its similarity to Marquis in dough handling quality. No. 14 was liked by chemists who favor a wheat with a mellow type of gluten. While not up to the standard of all-round strength expected of high-grade western Canadian wheat, it was regarded as a variety of good quality considering its protein level.

The curve for loaf volume on mixing time classed No. 14 as a medium-strength wheat closely resembling Marquis in its mixing requirements and mixing tolerance. Its quality was considered to be distinctly better than its protein content would indicate. But because of its lower protein level it was felt that millers might object to it on the grounds that it would not meet all the requirements of a bakers' grade of flour. It was classed as definitely inferior to Thatcher and last in baking strength. Its evaluation by the curve for loaf volume on mixing time is in good agreement with the general opinions of the majority of the collaborators.

Discussion

From a consideration of the three methods for evaluating the over-all strength and mixing characteristics of varieties, it is apparent that curves for loaf volume on mixing time are more informative, more readily interpreted, and more convincing than mixograms or farinograms. Moreover, as the over-all shape of the curve appears to be a varietal characteristic, it should prove most useful for typing varieties that are similar or dissimilar to the chosen standard in all-round baking behavior. Whether mixograms are superior to farinograms for indicating the strength and mixing characteristics of varieties is a matter of opinion, for the results of this study show that they do not always confirm each other. That they do not always agree with the curves for loaf volume on mixing time is not altogether surprising because physical dough curves are usually made from flour-water doughs, and curves for loaf volume on mixing time are made from fermented doughs carried through to the final loaf. This opinion is shared by Markley and Bailey (1939) who state that recording dough mixers give information that is distinct from that given by baking tests involving variations in mixing time, because changes in the mobility of doughs during fermentation are caused by factors other than those responsible for slackening during prolonged mixing. The agreement among the three sets of curves for classifying varieties that were similar in type to the two standard varieties is not particularly good in this particular study. As Marquis type varieties: the curves for loaf volume on mixing time included Nos. 10, 11, 12, and 14; the mixograms included Nos. 8, 10, 11, 12, and Regent; and the farinograms included Nos. 6, 9, and Regent. As Thatcher type varieties: the curves for loaf volume on mixing time included Nos. 4, 6, and Regent; the mixograms included Nos. 4 and 11; and the farinograms included Nos. 2, 4, 10, and 11.

In considering the opinions of the 19 collaborators who had occasion to test six varieties grown in 1944, it must be remembered that very few, if any, used exactly the same baking formulas and procedures. Furthermore, Canadian wheat is purchased for using alone and for blending with other wheats in foreign as well as in domestic markets, and the desired standards of quality naturally vary. In these circumstances, a new variety of wheat may be liked by one chemist for certain qualities and disliked by another chemist for the same qualities. This was brought to light in this study by the opinions expressed on Nos. 8 and 12. No. 8 was favored by one chemist for its shorter than normal spring wheat dough mixing requirements, because his mill catered to a market where flour with this characteristic was in demand. Another chemist disliked this variety for its shorter mixing and narrow fermen-

tation tolerance, because his mill exported large quantities of flour to countries where flours of wide mixing and fermentation characteristics are in common use. No. 12 appealed to one chemist because its pliable dough handling properties indicated that it would go through make-up machinery with considerable tolerance to variables that normally occur. Another chemist condemned No. 12 because its softer than normal dough properties were characteristic of soft winter wheats. All things considered, the opinions expressed and the mean placings of the samples are quite encouraging with respect to the interpretation of curves for loaf volume on mixing time.

It seems to be well recognized that flours possessing wide mixing tolerance also possess wide fermentation tolerance. Aitken and Geddes (1934), in their study of the behavior of flours of varying protein content when subjected to normal and severe baking procedures, concluded that increasing mixing time had the same general effect on loaf volume as increasing fermentation time. Markley (1937) also found a close relation between mixing and fermentation time. He observed a negative correlation between mixing and fermentation for the production of optimum bread and interpreted this to mean that, within certain limits, one can replace the other. This is confirmed by Stamberg and Bailey (1938) who found that mixing and fermentation tend to have an inversely related compensating effect.

In the light of these opinions it seems that the interpretation of curves for loaf volume on mixing time, as applied to mixing tolerance, can also apply to fermentation tolerance. The curve for loaf volume on mixing time offers considerable promise as a method for use in routine testing of Plant Breeders' Varieties, for not only does it provide useful information on the mixing requirements, mixing tolerance, and over-all strength of a flour, but, within certain limits, it serves to indicate fermentation tolerance also.

Summary

A method employing a differential mixing technique, in which loaves are baked from seven doughs, loaf volumes are plotted against mixing time, and a smooth curve is fitted to the points, has been used in studies of wheat varieties grown at several Canadian stations in 1943 and 1944. The curves are evaluated to show: over-all strength of the flour; extra or reserve strength; mixing requirements; and mixing tolerance. The curves can be readily compared, and offer convincing evidence of differences between varieties.

Comparison of curves for corresponding varieties grown in different years indicates that the curve for loaf volume on mixing time is a

varietal characteristic and, as such, serves to type varieties that are similar or dissimilar to accepted standard varieties. Intervarietal comparisons can best be made when the range in protein content is not unduly large; for, within a variety, the over-all level of the curve falls and tends to flatten with decreasing protein content.

Comparison of curves for loaf volume on mixing time with mixograms and farinograms shows that for indicating strength and mixing characteristics the curves for loaf volume on mixing time are more informative, more convincing, and more readily and objectively interpreted. The mixograms and farinograms do not always confirm each other nor do they always agree with the curves for loaf volume on mixing time. Agreement among the three sets of curves for classifying varieties that are similar in type to accepted standard varieties is only fairly good.

Acknowledgments

The authors gratefully acknowledge the suggestions and constant interest of Dr. J. Ansel Anderson, Chief Chemist of the laboratory, during the studies and in the preparation of the manuscript. The authors are indebted to J. G. Darroch, Agricultural Scientist, Dominion Rust Research Laboratory, Winnipeg, who made the statistical computations for the preparation of the smooth curves.

Literature Cited

Aitken, T. R., and Geddes, W. F.
1934 The behaviour of strong flours of widely varying protein content when subjected to normal and severe baking procedures. Cereal Chem. **11**: 487–504.

Geddes, W. F., Aitken, T. R., and Fisher, M. H.
1940 The relation between the normal farinogram and the baking strength of western Canadian wheat. Cereal Chem. **17**: 528–551.

Johnson, J. A., Swanson, C. O., and Bayfield, E. G.
1943 The correlation of mixograms with baking results. Cereal Chem. **20**: 625–644.

Larmour, R. K., Working, E. B., and Ofelt, C. W.
1939 Quality tests on hard red winter wheats. Cereal Chem. **16**: 733–752.

Markley, M. C.
1937 The scientific basis for experimental baking tests. Cereal Chem. **14**: 834–840.

—— and Bailey, C. H.
1939 The colloidal behavior of flour doughs. V. Comparison of the increase in mobility of doughs upon either prolonged mixing or fermentation with the effects of varied mixing times upon loaf characteristics. Cereal Chem. **16**: 265–271.

Munz, E., and Brabender, C. W.
1940 Prediction of baking value from measurements of plasticity and extensibility of dough. I. Influence of mixing and molding treatments upon physical dough properties of typical American wheat varieties. Cereal Chem. **17**: 78–100.

Stamberg, O. E., and Bailey, C. H.
1938 Relationship of mixing speed to dough development. Cereal Chem. **15**: 739–748.

Swanson, C. O., and Johnson, J. A.
1943 Description of mixograms. Cereal Chem. **20**: 39–42.

EFFECTS OF VARIETY AND ENVIRONMENT ON THE AMYLASES OF GERMINATED WHEAT AND BARLEY [1]

ERIC KNEEN and H. L. HADS [2]

Department of Agricultural Chemistry,
Nebraska Agricultural Experiment Station, Lincoln, Nebraska

(Received for publication January 23, 1945)

It is well established that the diastatic power of barley malt is influenced both by the variety of barley malted and by the environment in which it is grown. As examples may be cited the publications of Bishop (1936), Myrbäck (1936), Shellenberger and Bailey (1936), Hind (1938), Hills and Bailey (1938), and Dickson and co-workers (1938, 1939, 1940). The extensive Canadian research is summarized by Anderson, Sallans, and Meredith (1941) and by Anderson, Meredith, and Sallans (1943). These latter workers likewise reported that the starch liquefying (α-amylase) activity of barley malts was dependent on variety and environment. Burkert and Dickson (1941) demonstrated that the malts of two barley varieties varied widely in both α- and β-amylase, and in a further study of the individual components Nelson and Dickson (1942) showed them to be dependent on barley variety and on environment of growth.

Limited data are available for wheat. Geddes, Hildebrand, and Anderson (1941) found the amylolytic properties of malted wheat flours to be dependent both on type and protein content of the wheat. Meredith, Eva, and Anderson (1944) showed that the α- and β-amylase activities of malted wheat flours from Canadian hard red spring wheats were influenced by the variety and source of the wheat. An influence of wheat class on the development of the amylase components in malting has been demonstrated by Geddes and co-workers (private communication). Confining investigations solely to the ungerminated wheat, Elizarova (1940) reported that both the free and total β-amylase activities of spring wheat were higher than those of winter wheat, and Dadswell and Wragge (1940) showed a wide variation in the water-soluble β-amylase contents of a number of Australian bread flours.

The above indicates that, while probably adequate for barley, the data relating variety and environment to the amylases of wheat could well be supplemented. Accordingly, an investigation of the influence of grain source and variety on the amylases of wheat and germinated wheat was undertaken. The results of a study of barley are included primarily to demonstrate the applicability of the methods employed.

[1] Published with the approval of the Director as paper No. 369, Journal Series, Nebraska Agricultural Experiment Station.
[2] In service with the armed forces.

Methods

The methods used for germination and analysis essentially were those described in previous studies (Kneen, Miller, and Sandstedt, 1942; Kneen, 1944). Sound grain was steeped for 24 hours in distilled water at room temperature. Moisture determinations were not made before transferring the samples to the germination chamber. Germination was in the "rag dolls" previously described. Duration and temperature of germination are indicated in the discussion of the individual experiments. The rag dolls were maintained at near saturation throughout the germinations and an attempt was made to insure uniformity within an experiment. Following germination the samples were transferred to tables and dried under fans for 48 hours at room temperature.

For the determination of amylase activities both "free" and total (papain) extractions were made. All extractions were made at 30°C with dilute calcium acetate solution (1 mg per ml). When papain was used, it was at the 10% level. Extraction times varied from 15 minutes to 18 hours as indicated. The β-amylase activities of the germinated and ungerminated samples were determined by the method of Kneen and Sandstedt (1941); i.e., they represent the starch saccharification by the extracts less those amounts that could be calculated as due to the action of α-amylase. The α-amylase activities of the germinated samples were determined by the method of Sandstedt, Kneen, and Blish (1939), and represent therefore the rates of starch dextrinization effected by the extracts in the presence of an added excess of β-amylase. The modification of the dextrinization procedure proposed by Kneen, Sandstedt, and Hollenbeck (1943) was used to evaluate the small amounts of α-amylase present in the ungerminated grains. Here likewise the influence of variable β-amylase activity is eliminated by the addition of an excess of this enzyme. The degree of starch dextrinization achieved by prolonged action of the extract is evaluated by the corresponding reduction in the time necessary for a standard malt extract to complete the conversion.

The terms "free" and "total" amylase have their customary connotations: "free" amylase is that fraction readily extractable without the influence of added proteolytic enzyme, and "total" amylase is that amount appearing in the extract after extensive treatment with papain. The "free" amylase, in particular, is an indefinite fraction, its magnitude depending on the time and temperature of extraction and on the salt content of the extractant.

Barley Studies

For the barley investigations four varieties were used: Spartan, a two-rowed variety, and the six-rowed varieties Trebi, Club Mariout, and Flynn. They were produced during the season of 1941 at three Nebraska locations, Lincoln, North Platte, and Alliance, and under comparable conditions at each station. Samples of these barleys were germinated for 4 days at 18°C following the preliminary 24-hour steep at room temperature. Table I gives the data for the free and total

TABLE I

β-AMYLASE ACTIVITIES OF GERMINATED AND UNGERMINATED BARLEY

Source	Variety	Nitrogen content (barley)	β-amylase activities			
			Ungerminated barley		Germinated barley	
			"free" (1-hr)	total (papain)	"free" (1-hr)	total (papain)
		%	*units*	*units*	*units*	*units*
Lincoln	Spartan	3.06	7.7	23.5	19.6	25.2
Lincoln	Trebi	2.93	13.1	37.8	32.2	39.4
Lincoln	Club Mariout	2.88	12.3	33.0	17.6	32.0
Lincoln	Flynn	2.95	9.4	24.4	17.6	26.5
	Average	2.96	10.6	29.7	21.8	30.8
North Platte	Spartan	2.84	7.4	21.6	15.7	19.2
North Platte	Trebi	2.77	14.1	36.2	27.0	33.6
North Platte	Club Mariout	2.55	10.8	30.0	20.8	28.4
North Platte	Flynn	2.57	8.5	25.2	14.1	24.0
	Average	2.68	10.2	28.0	19.4	26.3
Alliance	Spartan	2.80	9.3	22.0	16.1	22.4
Alliance	Trebi	2.54	11.9	32.9	22.6	34.1
Alliance	Club Mariout	2.55	10.7	26.0	17.4	25.0
Alliance	Flynn	2.30	8.6	19.4	12.7	19.8
	Average	2.55	10.1	25.1	17.2	25.3

β-amylase activities of the germinated and ungerminated samples and for the nitrogen contents of the barleys.

The relationships shown in Table I for the various activities are in good general agreement with the data reported in the literature (e.g., Anderson and co-workers, 1941). The interstation relationship found between the β-amylase activities of the germinated barleys and either the total barley β-amylase or the barley nitrogen would be anticipated. Similarly, the intervarietal relationship between germinated barley β-amylase and barley total β-amylase and the lack of intervarietal relationship between β-amylase activities and barley nitrogen are in

agreement. The indication is that the inherent β-amylase relationships are brought out by the methods used in a similar manner to those found by conventional malting procedures.

The data found for the free α-amylase of the ungerminated barley and the free and total α-amylase activities of the germinated samples are given in Table II. The average sprout length at the end of the growth period is given for comparison. As noted previously (Kneen,

TABLE II

α-AMYLASE ACTIVITIES OF GERMINATED AND UNGERMINATED BARLEY

Source	Variety	Sprout length (germinated barley)	α-amylase activities		
			Ungerminated barley	Germinated barley	
			"free"	"free" (1-hr)	total (papain)
		mm	*units*	*units*	*units*
Lincoln	Spartan	30	0.012	76.6	76.6
Lincoln	Trebi	33	0.018	77.3	77.3
Lincoln	Club Mariout	28	0.025	74.2	76.3
Lincoln	Flynn	25	0.009	57.1	58.3
	Average	29	0.016	71.3	72.1
North Platte	Spartan	33	0.091	66.9	67.0
North Platte	Trebi	33	0.045	45.9	45.2
North Platte	Club Mariout	28	0.023	44.2	49.9
North Platte	Flynn	25	0.009	32.5	33.3
	Average	30	0.042	47.4	48.9
Alliance	Spartan	25	0.090	52.9	52.9
Alliance	Trebi	25	0.055	42.9	44.2
Alliance	Club Mariout	25	0.110	32.2	33.0
Alliance	Flynn	25	0.027	33.9	33.2
	Average	25	0.071	40.5	40.8

1945), sprout development is much more pronounced in rag dolls than, for example, in a drum-malting procedure.

The α-amylase activities of the germinated barleys follow the trend that would be expected: an interstation relationship with germinated barley β-amylase, barley total β-amylase, and barley nitrogen. No similar data for the α-amylase activity of ungerminated barley are available for comparison. From Table II it is apparent that there is little intervarietal relationship between barley α-amylase and the activity of the germinated samples. Any interstation relationship would appear to be inverse. However, the number of samples is much too few to draw any conclusions in this regard.

The results of the foregoing investigation with barley indicate that the type of information regarding amylase relationships given by the methods employed was essentially the same as that found by other workers. It may be concluded that such germination procedures and methods of analysis bring out amylase relationships comparable to those derived from the use of standard laboratory malting techniques. Accordingly, the same type of procedure was employed to investigate some varietal and environmental effects on the amylases of germinated and ungerminated hard red winter wheats.

Wheat Studies

Two studies were made with wheat, one using two varieties from six widely separated locations, the other using six varieties from two Nebraska locations. The samples were all harvested in the 1940 season and germination and analyses carried out early in 1941.

Influence of Environment on the Amylases of Wheat. For this investigation the varieties of hard red winter wheat, Nebred and Blackhull, were selected from samples grown at Beaverlodge and Lethbridge in Alberta, Canada, and from those grown at Moccasin, Montana, Fort Collins, Colorado, North Platte, Nebraska, and Lincoln, Nebraska. Germination was for 11 days at 10°C. Analyses were in the customary manner. For the ungerminated samples free α- and β-amylase activities were determined (1-hour calcium acetate extractions) and total β-amylase (18-hour extracts with papain). With the germinated samples three types of extracts were compared: 15-minute and 3-hour calcium acetate extracts and an 18-hour extraction with papain. The results found for *β-amylase* activities and for wheat nitrogen are presented in Table III.

Considering the data given in Table III it is apparent that there is a definite association between wheat total β-amylase and wheat nitrogen: the correlation coefficient of + .922 is above the 1% point. As with barley, the environmental factors contributing to the development of high nitrogen in winter wheat likewise resulted in high total β-amylase but not necessarily in high free β-amylase activity.

The β-amylase activities of the germinated samples, as obtained by any of the three methods of extraction, were related to the total β-amylase activities of the ungerminated grains and, accordingly, to the nitrogen content of the wheats. The correlation coefficients determined, those between germinated wheat total β-amylase and either wheat total β-amylase or wheat nitrogen were both above the 1% point.

The influence of environment on the *α-amylase* activities of germinated and ungerminated wheat is shown in Table IV. It is apparent

that the α-amylase activities of the germinated wheats were not so dependent on growth environment as were the β-amylase activities. A very loose association may exist between the total α-amylase and total β-amylase values of the germinated samples and between germinated wheat total α-amylase and either wheat nitrogen or wheat total β-amylase. The correlation coefficients are not statistically significant but might become so if a larger number of samples were

TABLE III

INFLUENCE OF GROWTH ENVIRONMENT ON β-AMYLASE ACTIVITIES OF GERMINATED AND UNGERMINATED HARD RED WINTER WHEAT

Variety	Source	Nitrogen content (wheat)	β-amylase activity				
			Ungerminated wheat		Germinated wheat		
			"free" (1-hr)	total (papain)	"free"		total (papain)
					15-min	3-hr	
		%	*units*	*units*	*units*	*units*	*units*
Nebred	Lincoln	3.55	7.5	28.6	22.4	27.2	30.0
Nebred	Ft. Collins	3.14	7.3	26.5	19.4	21.4	27.7
Nebred	Beaverlodge	2.97	7.2	26.7	22.9	22.6	25.0
Nebred	North Platte	3.00	6.2	25.6	22.0	24.3	25.3
Nebred	Moccasin	2.81	5.5	22.4	16.5	18.2	20.9
Nebred	Lethbridge	2.35	6.5	20.1	13.6	16.3	17.1
	Average	2.97	6.7	25.0	19.5	21.7	24.3
Blackhull	Lincoln	3.34	9.6	23.6	16.3	21.3	26.0
Blackhull	Ft. Collins	3.11	7.6	21.0	17.2	18.1	24.6
Blackhull	Beaverlodge	2.91	9.0	22.2	18.1	19.6	19.4
Blackhull	North Platte	2.83	7.2	20.9	16.9	17.1	20.5
Blackhull	Moccasin	2.94	7.0	19.6	13.8	14.6	18.8
Blackhull	Lethbridge	2.33	7.7	17.2	12.6	13.1	16.2
	Average	2.91	8.0	20.8	15.8	17.3	20.9

Correlation coefficients:
Wheat nitrogen × wheat total β-amylase = + .922.
Wheat nitrogen × germinated wheat total β-amylase = + .955.
Wheat total β-amylase × germinated wheat total β-amylase = + .918.

analyzed. Further, there is no pronounced relationship between the α-amylase activities of the germinated samples and those of the wheats. It may be concluded that the growth environment of the wheat does have an influence on the α-amylase activity of the germinated grain; with both varieties the Lincoln samples were high and the Moccasin samples low. This activity must be influenced by factors other than, or in addition to, those regulating the β-amylase activity.

Influence of Variety on the Amylases of Wheat. Six varieties of hard red winter wheat were selected from two uniform testing plots located

in the state. The same six were taken from Saunders County in eastern Nebraska and from Chase County in western Nebraska. Germination was for 10 days at 10°C. Analyses were made in the customary manner. With the ungerminated samples the "free" β-amylase activity was determined in 3-hour extractions. Both α- and β-amylase activities were evaluated in 18-hour papain extractions. For the germinated samples the α- and β-amylase activities of two extracts were compared: a 15-minute extraction and an 18-hour

TABLE IV

INFLUENCE OF GROWTH ENVIRONMENT ON α-AMYLASE ACTIVITIES OF GERMINATED AND UNGERMINATED HARD RED WINTER WHEAT

Variety	Source	Sprout length (germinated wheat)	Ungerminated wheat	Germinated wheat		
			"free"	"free"		total (papain)
				15-min	3-hr	
		mm	*units*	*units*	*units*	*units*
Nebred	Lincoln	35	0.010	249	261	264
Nebred	Ft. Collins	35	0.030	187	200	216
Nebred	Beaverlodge	38	0.024	216	230	235
Nebred	North Platte	38	0.011	214	233	233
Nebred	Moccasin	35	0.012	180	183	183
Nebred	Lethbridge	40	0.041	190	206	209
	Average	37	0.021	206	219	223
Blackhull	Lincoln	40	0.013	209	217	226
Blackhull	Ft. Collins	40	0.029	199	202	199
Blackhull	Beaverlodge	38	0.024	180	180	182
Blackhull	North Platte	38	0.033	187	189	184
Blackhull	Moccasin	35	0.015	162	165	167
Blackhull	Lethbridge	38	0.029	183	183	184
	Average	38	0.024	187	189	190

Correlation coefficients:
Wheat nitrogen × germinated wheat total α-amylase = + .667.
Germinated wheat total β-amylase × germinated wheat total α-amylase = + .759.
Wheat total β-amylase × germinated wheat total α-amylase = + .763.

papain extraction. The results for *β-amylase* activities are given in Table V.

The data of Table V indicate that there was a varietal influence on the β-amylase activities of the wheats either germinated or not, though the variation between varieties was not great. For the ungerminated wheats Nebred was highest in total β-amylase and Blackhull lowest. The same relationship held when the average germinated wheat total β-amylase values for the two counties were compared. No intervarietal relationship between amylase activity and wheat

nitrogen is apparent. However, as with the data of Table III, a strong interstation relationship is present; the Saunders County samples were higher in nitrogen and in all β-amylase activities than those from Chase County.

The influence of variety on the *α-amylase* activities of the wheats is shown by the data of Table VI. No dependence of the α-amylase activities of the ungerminated wheats on variety is apparent; the values found were exceedingly uniform throughout. On the other hand, the α-amylase activities of the germinated samples proved to be dependent

TABLE V

INFLUENCE OF VARIETY ON β-AMYLASE ACTIVITIES OF GERMINATED AND UNGERMINATED HARD RED WINTER WHEAT

Source	Variety	Nitrogen content (wheat)	β-amylase activity			
			Ungerminated wheat		Germinated wheat	
			"free" (3-hr)	total (papain)	"free" (15-min)	total (papain)
		%	*units*	*units*	*units*	*units*
Saunders County	Nebred	3.67	7.4	28.0	20.0	25.8
Saunders County	Cheyenne	3.38	9.7	26.5	19.1	24.7
Saunders County	Turkey	3.67	9.0	26.6	17.9	24.9
Saunders County	Tenmarq	3.57	9.3	25.9	19.5	26.1
Saunders County	Chiefkan	3.56	8.1	24.4	18.3	24.0
Saunders County	Blackhull	3.43	9.0	21.9	16.2	21.6
	Average	3.55	8.8	25.6	18.5	24.5
Chase County	Nebred	2.82	6.8	23.3	16.4	21.6
Chase County	Cheyenne	2.79	7.3	22.9	16.6	22.1
Chase County	Turkey	2.90	7.7	21.8	16.2	21.1
Chase County	Tenmarq	2.81	7.1	21.8	17.4	20.6
Chase County	Chiefkan	2.82	6.5	19.1	17.7	18.1
Chase County	Blackhull	2.94	7.3	19.5	11.6	16.7
	Average	2.85	7.1	21.4	16.0	20.0

on variety, and in a similar manner to the β-amylase activities. Again Nebred averaged highest and Blackhull lowest of the six varieties. Further, an environmental influence is evident inasmuch as the high nitrogen, high β-amylase samples from Saunders County likewise showed high α-amylase activities when germinated.

Discussion

A consideration of the studies with wheat shows that, as with barley, both variety and environment are factors influencing the α- and β-amylase activities of the germinated samples. It would be anticipated that similar relationships would pertain under conventional

malting procedures. The limited data indicate that, within the hard red winter class, wheats grown in an environment conducive to the production of high nitrogen grain tend to have a high total β-amylase content and therefore a high β-amylase activity when malted. Such wheats likewise may have somewhat superior ability for the elaboration of α-amylase activity by malting. The indications are that no prediction of the α-amylase activity of the malted wheat could be made from an evaluation of the relatively minute amounts of this enzyme in the ungerminated grain. Certain varieties appear to be definitely superior

TABLE VI

INFLUENCE OF VARIETY ON α-AMYLASE ACTIVITIES OF GERMINATED AND UNGERMINATED HARD RED WINTER WHEAT

Source	Variety	Sprout length (germinated wheat)	α-amylase activity		
			Ungerminated wheat	Germinated wheat	
			"free"	"free" (15-min)	total (papain)
		mm	*units*	*units*	*units*
Saunders County	Nebred	25	0.031	221	239
Saunders County	Cheyenne	33	0.036	204	223
Saunders County	Turkey	25	0.030	190	229
Saunders County	Tenmarq	28	0.041	171	207
Saunders County	Chiefkan	30	0.031	177	207
Saunders County	Blackhull	25	0.042	156	179
	Average	28	0.035	187	214
Chase County	Nebred	30	0.040	177	191
Chase County	Cheyenne	25	0.036	148	171
Chase County	Turkey	33	0.036	166	178
Chase County	Tenmarq	35	0.043	161	184
Chase County	Chiefkan	30	0.040	140	195
Chase County	Blackhull	30	0.031	133	157
	Average	31	0.038	154	179

to others for the development of amylase activity in malting, though the variation in this respect was not found to be great.

The values given in the tables for the α-amylase activities of the ungerminated grains indicate that, far from being absent, this enzyme is present in appreciable and variable quantities. For the barleys, the variation in α-amylase activity was from 0.009 units to 0.11 units, and for the wheats from 0.01 units to 0.043 units. Other data (unpublished) have shown that ungerminated wheats and sound wheat flours may have considerably more α-amylase activity than indicated by this latter figure. When it is considered that the addition of as much as

0.2% of an average malt to an α-amylase-free wheat flour would raise that flour to an α-amylase activity of no more than 0.1 to 0.2 units, it may well be that the small and variable amounts of this enzyme in wheat flours have considerable significance.

The data given in the tables would indicate that wheat is much superior to barley in its capacity to produce α-amylase during germination. No such direct comparison is justified. The barleys were germinated for a short period of time at a relatively high temperature and the wheats for a longer period at a low temperature. Further, the differences may have been emphasized by the type of germination employed and by the varieties of the two cereals selected for study. It has been shown by Geddes and co-workers (private communication) that the α-amylase activities of wheats malted under conventional procedures are of the same order as those of malted barley. Any valid comparison of wheat and barley would necessarily require a much more extensive study than the one made here, both with regard to the number of samples and the response to varied malting conditions.

Summary

Four varieties of barley from three locations were germinated by a rag-doll procedure and amylase activities determined. The relationships found conformed well with those reported in the literature. The β-amylase activities of the ungerminated grains and the α- and β-amylase activities of the germinated samples were influenced both by variety and by environment of growth. There was an interstation relationship between barley nitrogen, barley total β-amylase, and the α- and β-amylase activities of the germinated samples.

Similar germination and analytical techniques were used to investigate the amylase activities of two hard red winter wheat varieties from six locations and six varieties from two locations. With this cereal too, the β-amylase activities of the ungerminated grain and the α- and β-amylase activities of the germinated samples were dependent on both variety and environment of growth. There was an interstation relationship between wheat nitrogen and wheat total β-amylase and between either of these properties and the β-amylase activities of the germinated samples. On an interstation basis, a tendency for those germinated samples having high total β-amylase also to have high α-amylase activity was evident. No relationship was apparent between the α-amylase activities of the ungerminated wheats and any other property, either of the ungerminated or the germinated samples.

With hard red winter wheats, as with barley, a prominent factor in the selection of samples for the development of α- and β-amylase by

malting should be the environment of growth. The indication is that wheats from locations conducive to the production of high-nitrogen grain would be superior in this respect. Among those grown at any one location, a further selection may be made on the basis of variety.

Acknowledgment

The assistance rendered by the Stations at Beaverlodge and Lethbridge, Alberta, and at Moccasin, Montana, and Fort Collins, Colorado, in making samples available is gratefully acknowledged. In addition, thanks are due Dr. K. S. Quisenberry of the Nebraska Station for assistance in the collection of samples.

Literature Cited

Anderson, J. A., Meredith, W. O. S., and Sallans, H. R.
1943 Malting quality of Canadian barleys. IV. A summary of information of special interest to plant breeders. Sci. Agr. **23**: 297–314.
——, Sallans, H. R., and Meredith, W. O. S.
1941 Varietal differences in barleys and malts. XII. Summary of correlations between 18 major barley, malt, and malting properties. Can. J. Research C **19**: 278–291.
Bishop, L. R.
1936 Effect of variety, soil and season on the barleys and malts of the seasons 1932 and 1933. J. Inst. Brewing **42**: 10–14.
Burkert, G. M., and Dickson, A. D.
1941 The action of the amylases of two barley varieties on the barley starches. Cereal Chem. **18**: 605–615.
Dadswell, I. W., and Wragge, W. B.
1940 The autolytic digestion of flour in relation to variety and environment. Cereal Chem. **17**: 584–601.
Dickson, J. G., Dickson, A. D., Shands, H. L., and Burkhart, B. A.
1938 Barley and malt studies. IV. Experimental malting of barleys grown in 1936 and summary data for three years 1934–1936. Cereal Chem. **15**: 133–168.
1940 Barley and malt studies. VI. Experimental malting of barleys grown in 1938. Cereal Chem. **17**: 495–504.
——, Shands, H. L., Dickson, A. D., and Burkhart, B. A.
1939 Barley and malt studies. V. Experimental malting of barleys grown in 1937. Cereal Chem. **16**: 468–480.
Elizarova, S. S.
1940 The β-amylase of wheat and barley. Compt. rend. acad. sci. U.R.S.S. **26**: 698–701.
Geddes, W. F., Hildebrand, F. C., and Anderson, J. A.
1941 The effect of wheat type, protein content, and malting conditions on the properties of malted wheat flour. Cereal Chem. **18**: 42–60.
Hills, C. H., and Bailey, C. H.
1938 The amylases of different barley varieties. Cereal Chem. **15**: 351–362.
Hind, H. L.
1938 Interpretation of the analyses of Indian barleys and malts. J. Inst. Brewing **44**: 559–562.
Kneen, E.
1944 A comparative study of the development of amylases in germinating cereals. Cereal Chem. **21**: 304–314.
1945 Sorghum amylase. Cereal Chem. **22**: 112–134.
——, Miller, B. S., and Sandstedt, R. M.
1942 The influence of temperature on the development of amylase in germinating wheat. Cereal Chem. **19**: 11–27.
—— and Sandstedt, R. M.
1941 Beta-amylase activity and its determination in germinated and ungerminated cereals. Cereal Chem. **18**: 237–252.
——, ——, and Hollenbeck, C. M.
1943 The differential stability of the malt amylases—separation of the alpha and beta components. Cereal Chem. **20**: 399–423.

Meredith, W. O. S., Eva, W. J., and Anderson, J. A.
1944 Effect of variety and environment on some qualities of malted wheat flour. Cereal Chem. **21**: 233–240.
Myrbäck, K.
1936 Über den Amylasegehalt von Gerste reiner Linien. Enzymologia **1**: 280–287.
Nelson, D. H., and Dickson, A. D.
1942 The application of the Kneen and Sandstedt methods for alpha- and beta-amylase to malts from different barley varieties. Am. Soc. Brew. Chem., Proc. **5, 1942**: 61–67.
Sandstedt, R. M., Kneen, E., and Blish, M. J.
1939 A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16**: 712–723.
Shellenberger, J. A., and Bailey, C. H.
1936 Biochemical distinctions between barley varieties. Cereal Chem. **13**: 631–655.

EFFECT OF MOISTURE CONTENT, TEMPERATURE, AND LENGTH OF STORAGE ON THE DEVELOPMENT OF "SICK" WHEAT IN SEALED CONTAINERS [1]

EDWARD P. CARTER and GEORGE Y. YOUNG [2]

(Received for publication June 23, 1944)

A type of damage in stored wheat known in the trade as "sick" wheat has been recognized since about 1921. It occurs both in terminal and country elevators, especially in the former, but is rarely found in farm storage.[3] A unique feature is its occurrence without obvious heating of the grain such as usually takes place when damp grain is stored in large quantities.

The grain trade defines "sick" wheat as a type of damage characterized by kernels having a dull, lifeless appearance (other than "Tombstone") which may be accompanied by a moldy appearance of the germ or by mold in the crease. This condition may be associated with a discoloration of the body of the kernel resembling slightly heat-damaged grain. "Sick" kernels develop deadened germ ends, easily distinguished as damaged by their black or brownish appearance when the germ is exposed. This type of damage is sometimes accompanied by a musty odor. In the present paper the authors have used the definition of Geddes (1943) who has defined sick wheat as that "in which the seed loses its viability and the germ darkens in color and becomes rancid." The quality of "sick" wheat as shown by bread-

[1] The work reported herein was supported by Bankhead-Jones, S.R.F.-2-7, "Investigation of Grain Storage on the Farm," and was conducted in cooperation with the Division of Agricultural Engineering, of the Bureau of Plant Industry, Soils, and Agricultural Engineering, and the Grain Products Branch, Office of Distribution, War Food Administration, U. S. Department of Agriculture. Credit is due the Board of Grain Supervisors of the Grain Products Branch for the determination of "sick" wheat kernels.
[2] Assistant Pathologists, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.
[3] Private correspondence, H. F. Prue, Grain Supervisor, Grain Products Branch, War Food Administration, Office of Distribution, U. S. Department of Agriculture, Toledo, Ohio.

baking tests is distinctly poor, although better than that of heat-damaged grain.

The cause of "sick" wheat, as distinct from heat damage or other forms of damage of high moisture wheat in storage, is not known. It seems to occur only when wheat is stored in large quantities and makes its appearance only after a considerable time. Its occurrence in deep bins has suggested to some workers that anaerobic rather than aerobic respiration is a factor of primary importance. Coleman [4] thought that the "sick" condition might be caused by the by-products of slowly developing fermentation. He found that moderately moist wheat (15%) stored at temperatures between 70° and 85°F will develop a wet or dank odor sometimes associated with "sick" wheat. Thomas (1937) showed experimentally that certain fungi commonly found on sound grain may, under suitable conditions of temperature and moisture, elaborate by-products that are toxic to the wheat embryo and kill it. He called such wheat "sick" wheat, but whether it is identical with or similar to the "sick" wheat of commerce is not stated. Swanson (1926) found that the milling and baking qualities of dead wheat were poor, caused by a granular and brittle gluten. Kühl (1941), reporting on the effects of cereal fats and free fatty acids on the baking capacity of flour, stated that the addition of relatively large amounts of free fatty acids impaired the quality of the gluten. Swanson (1934) found that mold growth could be inhibited by the entire exclusion of air from the storage container. Moist wheat so stored showed little or no visible signs of damage, but viability, acidity, and baking quality were seriously affected. It was found that the period of storage was important, the severity of damage increasing with time in storage. Swanson states that "sick" wheat developed in many of the moist samples stored in sealed containers. Zeleny and Coleman (1938) showed that the determination of fat acidity (milligrams potassium hydroxide necessary to neutralize the free fatty acids in 100 g of grain [dry weight]) may be used as a good index of soundness and extent of deterioration in wheat and other cereals, being better in this respect than other methods for determining titratable acidity. This test has been used by Zeleny (1940) for determining the soundness of corn in experimental storage.

During an investigation of the causes of heating of moist wheat, it was observed that wheat in sealed vacuum flasks sometimes became sour without heating. Since this wheat resembled "sick" wheat in many ways it seemed desirable to investigate the matter further.

[4] Coleman, D. A. 1929. A suggested method for determining unsoundness in wheat or flour with some applications of the test. (Unpublished Report.) Grain Division, Bureau of Agricultural Economics, U. S. Department of Agriculture.

Experiments accordingly were undertaken in which wheat containing different quantities of moisture was stored in sealed quart glass jars at different temperatures in temperature-controlled cabinets in the laboratory for various periods of time and then examined for evidence of "sick" wheat. The purpose of this paper is to report the results of these experiments.

Materials and Methods

The experiments were largely exploratory and were not all carried out at the same time as would have been desirable. Since the grain was kept in temperature-controlled cabinets during the course of the observations, this defect is believed to be relatively unimportant. It should be noted, however, that the sub-lots were not all precisely alike as regards germination and fat acidity when storage was begun because of changes having taken place since the wheat had been purchased.

Soft red winter wheat of the Fulcaster type, Grade No. 1, was used in these experiments. This wheat when purchased germinated 90% or more and had a low fat acidity characteristic of sound wheat, but slight deterioration between date of purchase and time of use was found to have occurred in certain instances as will be noted later. The first study to be undertaken was with a subsample placed in the glass jars in the temperature-controlled cabinets on August 11, 1939. A second experiment was started September 30, 1939, and three others in May, June, and July, 1940. During the interval between these tests the wheat not then in use was stored in a cold room at a temperature of about 40°F (4.4°C). For each experiment the subsample was tempered by the addition of a calculated amount of water and allowed to stand for 36 hours, during which time it was stirred and mixed frequently to assure an even distribution of moisture. The conditioned grain was then placed in quart Mason jars, fitted with rubber sealing rings. The tops were screwed down tightly and sealed by inverting the jars and dipping the tops twice in melted paraffin. The jars were then placed in constant temperature cabinets maintained at temperatures ranging from 5° to 40°C, at 5° intervals. Three experiments were conducted with wheat containing 12.2, 16.2, and 18.6% moisture respectively at each temperature level and two were conducted with moisture contents of 14.9 and 15.4% at temperatures ranging from 5° to 25°C only. Usually four to eight jars of each moisture series were placed in each temperature cabinet.

After different periods of storage, the jars were removed from the cabinets, and the wheat examined for signs of fungus growth. Any jar containing wheat with visible mold growth was discarded. For each

sampling, one of the remaining jars from each temperature was opened and the contents of each thoroughly mixed and divided into two aliquots by passing through a grain divider three times. One aliquot was dried to about 8% moisture on top of a warm oven and then placed in seed envelopes for determination of "sick" or otherwise damaged wheat. The second aliquot was used for determinations of moisture content, germination, and fat acidity. That the jars were actually sealed airtight is indicated by the fact that the moisture content of the wheat at the end of each experimental storage period did not vary more than 0.1% from that at the start of the period.

The moisture content of the wheat was determined by drying in a vacuum oven for 12 days at 80°C. Germination tests of duplicate samples of 50 kernels each were made on moist blotters in petri dishes at 10°C. Fat acidity determinations were conducted in duplicate according to the method suggested by Zeleny and Coleman (1938) and by Zeleny (1939), the average of two determinations being reported as the number of milligrams of potassium hydroxide necessary to neutralize the free fatty acids from 100 g of grain (dry basis). "Sick" wheat is reported as the percentage of the kernels having the characteristic appearance of "sick" wheat as determined visually by the Board of Grain Supervisors, Grain Products Branch, War Food Administration, U. S. Department of Agriculture.

Experimental Results

The results obtained from storing the sealed wheat samples with 12.2, 14.9, 15.4, 16.2, and 18.6% moisture, at various temperatures for different periods of time, are given in Tables I to V, respectively. The tables are arranged in the order of ascending moisture content of the wheat used in each case. The beginning date of each storage period is indicated in the legends of the tables.

There were no visual symptoms of the "sick" condition in wheat with a moisture content of 12.2% (Table I) when stored at temperatures less than 40°C and then only after 279 days storage at which time 11% of the kernels were classified as "sick." At the end of 391 days when the experiment was discontinued, 40% of the kernels were so classified. The fat acidity determinations, however, increased at all temperatures. With temperatures above 20°C there was a marked increase in fat acidity especially with the longer period of storage. Viability was not materially affected with temperatures of less than 35°C and then only after prolonged (279 days) storage, but rapidly declined to zero when stored at 40°C. There is obviously a high positive correlation between the increase in fat acidity and percentage of

TABLE I

FAT ACIDITY, GERMINATION, AND "SICK" KERNELS IN WHEAT CONTAINING 12.2% MOISTURE STORED BEGINNING MAY 9, 1940, IN SEALED CONTAINERS AT VARIOUS TEMPERATURES AND FOR VARIOUS PERIODS OF TIME

Storage		Fat acidity		"Sick" kernels	Germination
Temperature	Time	Observed	Increment		
°C	*Days*	*Mg KOH*	*Mg KOH*	%	%
—	0	17.9	0.0	0	90
5	31	22.1	4.2	0	98
5	130	21.5	3.6	0	100
5	279	22.3	4.4	0	97
5	391	26.4	8.5	0	100
10	31	21.5	3.6	0	90
10	130	20.5	2.6	0	94
10	279	21.7	3.8	0	98
10	391	25.2	7.3	0	94
15	31	19.2	1.3	0	96
15	130	21.5	3.6	0	92
15	279	23.6	5.7	0	100
15	391	25.9	8.0	0	96
20	31	20.4	2.5	0	98
20	130	21.3	3.4	0	96
20	279	25.9	8.0	0	98
20	391	31.0	13.1	0	98
25	31	20.4	2.5	0	98
25	130	24.9	7.0	0	96
25	279	31.8	13.9	0	90
25	391	37.6	19.7	0	88
30	31	23.2	5.3	0	92
30	130	[1]	[1]	[1]	[1]
30	279	[1]	[1]	[1]	[1]
30	391	[1]	[1]	[1]	[1]
35	31	22.4	4.5	0	98
35	130	30.3	12.4	0	90
35	279	47.1	29.2	0	5
35	391	51.5	33.3	0	0
40	31	26.0	8.1	0	0
40	130	36.7	18.8	0	0
40	279	56.4	38.8	11	0
40	391	83.6	65.7	40	0

[1] Samples discarded because of faulty temperature control.

"sick" kernels and an equally obvious negative correlation between these and percentage germination.

The general behavior of the wheat containing 14.9% moisture (Table II) was much the same, except there appeared to be a slight tendency for "sick" wheat to be produced at lower temperatures, since

a small percentage of "sick" wheat was found also in the wheat stored at 15° and 20°C and a trace when stored at 10°C. No samples were stored at temperatures above 25°C and the periods of storage were not the same as for the wheat containing 12.2% moisture. At 25°C, 14% of the kernels exhibited visual symptoms of the "sick" condition after 357 days of storage with increasing percentages with increase in the

TABLE II

FAT ACIDITY, GERMINATION, AND "SICK" KERNELS IN WHEAT CONTAINING 14.9% MOISTURE STORED BEGINNING JUNE 15, 1940, IN SEALED CONTAINERS AT VARIOUS TEMPERATURES AND FOR VARIOUS PERIODS OF TIME

Storage		Fat acidity		"Sick" kernels	Germination
Temperature	Time	Observed	Increment		
°C	*Days*	*Mg KOH*	*Mg KOH*	%	%
—	0	23.1	0.0	0	92
5	33	20.6	−2.5	0	86
5	128	25.4	+2.3	0	94
5	357	31.7	8.6	0	92
5	493	30.2	7.1	0	90
10	33	27.4	4.3	0	88
10	128	27.0	3.9	0	92
10	357	27.6	4.5	trace	98
10	493	32.4	9.3	0	90
15	33	27.8	4.7	0	86
15	128	25.7	2.6	0	94
15	357	32.5	9.4	0	92
15	493	34.1	11.0	6	72
20	33	27.4	4.3	0	86
20	128	28.7	5.6	0	88
20	357	37.1	14.0	0	24
20	416	38.7	15.6	10	4
20	493	42.8	19.7	0	0
20	687	49.8	26.7	8	0
25	33	29.4	6.3	0	84
25	128	29.3	6.2	0	68
25	357	47.8	14.7	14	0
25	416	49.9	16.8	19	0
25	687	70.1	47.0	27	0

storage period. Fat acidity increased and germination decreased with temperature and storage period, the changes in both cases being somewhat more rapid and more pronounced than with wheat containing 12.2% moisture.

The percentage of "sick" kernels and the loss in viability of the wheat containing 15.4% moisture (Table III) were greater than for the 14.9% moisture wheat. The fat acidity at comparable tempera-

tures was about the same, but the increase was somewhat less owing to a higher level in the wheat at the beginning of the experiment. This experiment, it should be noted, was begun more than a year later and hence may not be strictly comparable with those discussed above.

One hundred percent of the kernels of all samples of the wheat containing 16.2% moisture (Table IV) stored at 35° and 40°C were

TABLE III

FAT ACIDITY, GERMINATION, AND "SICK" KERNELS IN WHEAT CONTAINING 15.4% MOISTURE STORED BEGINNING JULY 7, 1940, IN SEALED CONTAINERS AT VARIOUS TEMPERATURES AND FOR VARIOUS PERIODS OF TIME

Storage		Fat acidity		"Sick" kernels	Germination
Temperature	Time	Observed	Increment		
°C	*Days*	*Mg KOH*	*Mg KOH*	%	%
—	0	33.3	0.0	0	98
5	82	35.0	1.7	0	96
5	390	40.2	6.9	trace	92
5	466	30.5	−2.8	0	89
5	629	35.3	2.0	0	90
10	82	36.8	3.5	0	94
10	390	35.1	1.8	0	94
10	466	36.3	3.0	0	84
10	629	38.8	5.5	0	88
15	82	35.4	2.1	0	96
15	390	35.3	2.0	0	78
15	466	39.4	9.7	0	79
15	629	43.6	10.3	1	70
20	82	34.7	1.4	0	96
20	390	35.3	2.0	3.5	6
20	466	43.0	9.7	13	0
20	629	52.4	19.1	12	0
20	634	48.1	14.8	15	0
25	82	40.1	6.8	0	16
25	390	48.8	15.5	20	0
25	466	54.1	20.8	33	0
25	629	72.1	38.8	58	0
25	634	74.3	41.0	70	0

designated as "sick" at the end of 176 days of storage and the experiment was discontinued at the end of 210 days. Direct comparison of the results with those of Tables I, II, and III is not possible because of the difference in storage periods. It is apparent, nevertheless, that the percentage of "sick" kernels tends to be greater especially at the lower storage temperatures. The fat acidity increased with temperature and time as in the other experiments, but the increase appears not to have been materially greater than in the drier wheat.

TABLE IV

FAT ACIDITY, GERMINATION, AND "SICK" KERNELS IN WHEAT CONTAINING 16.2% MOISTURE STORED SEPTEMBER 30, 1939, IN SEALED CONTAINERS AT VARIOUS TEMPERATURES AND FOR VARIOUS PERIODS OF TIME

Storage		Fat acidity		"Sick" kernels	Germination
Temperature	Time	Observed	Increment		
°C	*Days*	*Mg KOH*	*Mg KOH*	%	%
—	0	21.1	0.0	0	52
5	59	16.7	− 4.4	1.5	58
5	124	22.2	+ 1.1	1.0	48
5	176	19.6	− 1.5	1.5	50
5	210	18.6	− 2.5	0.5	42
10	59	16.7	− 4.4	1.0	48
10	124	21.5	+ 0.4	2.0	54
10	176	20.7	− 0.4	0.5	46
10	210	20.1	− 1.0	3.0	28
15	59	18.3	− 2.8	0.5	50
15	124	22.9	+ 1.8	0	32
15	176	20.9	− 0.2	0	44
15	210	20.7	− 0.4	1.5	30
20	59	19.0	− 2.1	1.0	48
20	124	28.3	+ 7.2	0.0	30
20	176	25.6	+ 4.5	5.0	24
20	210	27.1	+ 6.0	1.5	4
25	59	21.0	− 0.1	0.0	44
25	124	31.1	+10.0	3.0	12
25	176	30.8	+ 9.7	1.5	6
25	210	35.0	+13.9	16.0	0
30	59	24.0	+ 2.9	4.0	18
30	124	38.5	+17.4	9.0	0
30	176	39.5	+18.4	17.0	0
30	210	44.0	+22.9	20.0	0
35	59	30.6	+ 9.5	18.0	0
35	124	45.5	+24.4	96.0	0
35	176	50.4	+29.3	100.0	0
35	210	52.8	+31.7	100.0	0
40	59	35.1	+14.0	100.0	0
40	124	51.4	+30.3	100.0	0
40	176	50.9	+29.8	100.0	0
40	210	[1]	[1]	[1]	[1]

[1] Sample discarded because of mold growth.

The low initial germination of the grain in this test was probably caused by treating the wheat with an excess of chloropicrin for weevil and moth control between the time the grain was received and the experiment begun, a period of about two months.

"Sick" wheat developed at all temperatures in the wheat containing

TABLE V

FAT ACIDITY, GERMINATION, AND "SICK" KERNELS IN WHEAT CONTAINING 18.6% MOISTURE STORED BEGINNING AUGUST 11, 1939, IN SEALED CONTAINERS AT VARIOUS TEMPERATURES AND FOR VARIOUS PERIODS OF TIME

Storage		Fat acidity		"Sick" kernels	Germination
Temperature	Time	Observed	Increment		
°C	*Days*	*Mg KOH*	*Mg KOH*	%	%
—	0	15.4	0.0	0	84
5	7	16.0	0.6	0	84
5	32	19.0	3.6	2	58
5	108	16.8	1.4	7	36
5	200	19.3	3.9	7	42
10	7	16.0	0.6	8	56
10	32	18.0	2.6	2	52
10	108	21.0	5.6	0	41
10	200	24.7	9.3	36	26
15	7	16.0	0.6	5	34
15	32	19.4	4.0	3	60
15	108	21.0	5.6	0	41
15	200	31.7	16.3	14	26
20	7	16.2	0.8	2	44
20	32	19.0	3.6	7	58
20	108	19.0	3.6	10	37
20	200	25.9	10.5	12	0
25	7	15.1	−0.3	1	28
25	32	20.6	+5.2	2	50
25	108	21.4	6.0	2.5	2
25	200	29.7	14.3	17	0
30	7	16.5	1.1	2	46
30	32	20.5	5.1	5	48
30	108	46.2	30.8	100	0
30	200	[1]	[1]	[1]	[1]
35	7	16.5	1.1	7	40
35	32	[1]	[1]	[1]	[1]
35	108	[1]	[1]	[1]	[1]
35	200	[1]	[1]	[1]	[1]
40	7	16.5	1.1	10	10
40	32	23.4	8.0	100	0
40	108	38.0	22.6	100	0
40	200	41.9	26.5	100	0

[1] Sample discarded because of mold growth.

18.6% moisture (Table V), reaching 100% when stored at 40°C. As for the wheat containing 16.2% moisture, the experiment was discontinued much earlier than the others, *i.e.*, after 200 days of storage. Also as for the 16.2% moisture wheat the increase in fat acidity and decrease in viability was somewhat irregular and no greater or perhaps

even less than for the drier wheat stored at comparable temperatures and for similar periods of time.

Considered as a whole these experiments clearly indicate that "sick" wheat, comparable in appearance, at least, to that observed in commerce, can be produced by storage in small quantities in sealed containers, and the proportion tends to increase with storage temperature, moisture content of the grain, and time. The fact that "sick" kernels can be produced in wheat containing as little as 12.2% moisture would appear to be of special interest since this is below the level often thought necessary for safe storage. It should be remembered, however, that in these experiments "sick" wheat was not observed in such dry wheat at storage temperatures of less than 40°C and then only after 279 days of storage.

As would be expected, the increase in the percentage of "sick" kernels in any one experiment was accompanied by an increase in fat acidity and a decrease in germination. However, the increase in "sick" kernels induced by greater moisture content of the grain appears not to have been so clearly correlated with an increase in fat acidity or a decrease in germination. In other words, increasing the moisture content of the grain increased the proportion of "sick" kernels but not the fat acidity. The decrease in germination appears to have been accelerated by increasing the moisture content of the grain, but the data are inconclusive because of differences in the original subsamples and noncomparability of storage periods.

The results suggest that the "sick" wheat condition is associated with anaerobic storage conditions, but do not point to the exact cause or causes of it. It is thought that the "sick" wheat may be produced by suffocation of the grain, resulting from the respiratory consumption of molecular oxygen in the surrounding air. However, the possibility of deterioration by fungal action before mold growth can be detected by the naked eye, as mentioned by Larmour, Sallans, and Craig (1944), should not be overlooked. Tervet (1945) has indicated that there is a strong and immediate effect of certain mold spores and cell-free staled culture medium on the germination of healthy (soybean) seed. Other possibilities which should be investigated are the effect of anaerobic respiration of the seeds with accompanying chemical changes in the substrate material and the production, through respiratory processes, of end products toxic to the embryo.

The relation of these results to the occurrence of "sick" wheat in commercial storage has not been determined. Obviously the conditions are quite different, especially as regards the quantities of wheat involved. The fact that "sick" wheat was produced artificially under

anaerobic conditions and without visible evidence of fungi appears to support the belief that the reduction in oxygen pressure in large storage bins is a contributing factor.

Summary

"Sick" wheat was produced artificially by storing sound wheat containing different amounts of moisture in sealed quart Mason jars at various temperatures in temperature-controlled cabinets, the proportion of "sick" kernels in general increasing with the moisture content of the grain, the temperature of storage, and the length of the storage period. There was no visible fungus growth on the wheat such as usually takes place when moist wheat is exposed in the open air, except in a few jars and these were discarded.

Wheat containing 12.2% moisture stored at 40°C developed "sick" wheat symptoms when stored 279 days or longer, but not when stored at a lower temperature. A small percentage of "sick" wheat was produced in 32 days in wheat containing 18.6% moisture when stored at 5°C, and up to 100% "sick" kernels when stored at higher temperatures and for longer periods of time. The development of the "sick" wheat condition in general was accompanied by loss in viability and increase in fat acidity.

Literature Cited

Geddes, W. F.
1943 Factors that affect keeping quality of grain in storage. Northwestern Miller **215** (6): 6a.

Kühl, H.
1941 The action of cereal fats and free fat acids on the baking capacity of flour with special reference to storage. Mehl u. Brot **41**: 297–298; Chem. Abst. **38**: 2130–2138, 1944. (Abstract only seen.)

Larmour, R. K., Sallans, H. R., and Craig, B. M.
1944 Respiration of whole and dehulled sunflowerseed and of flaxseed. Can. J. Res. **22** (F): 9–18.

Swanson, C. O.
1926 The milling and baking qualities of a dead wheat. Northwestern Miller **146**: 154.
1934 Some factors involved in damage to wheat quality. Cereal Chem. **11**: 173–199.

Tervet, I. W.
1945 The influence of fungi on storage, on seed viability and seedling vigor of soybeans. Phytopathology **35**: 3–15.

Thomas, R. C.
1937 The role of certain fungi in the "sick wheat" problem. Ohio Agr. Expt. Sta. Bimo. Bul. **22**: 43–45.

Zeleny, L.
1939 Report on acidity in flour. J. Assoc. Official Agr. Chem. **22**: 526–535.
1940 Fat acidity in relation to heating of corn in storage. Cereal Chem. **17**: 29–37.

—— and Coleman, D. A.
1938 Acidity in cereals and cereal products, its determination and significance. Cereal Chem. **15**: 580–595.

THIAMINE RETENTION AND COMPOSITION OF U. S. ARMY BREAD[1]

R. B. MECKEL and G. ANDERSON

American Institute of Baking, Chicago, Illinois

(Received for publication February 16, 1945)

Bread is recognized by the Army as a universally liked food of considerable nutritive value. For this reason the Army has from its earliest days devoted considerable attention to the training of troops to prepare good bread under both garrison and field conditions. A monograph written as long ago as 1882 by Major George Bell provides many interesting notes on flour and detailed instructions for the making of bread and for the construction and operation of permanent ovens and field ovens. The present day baking practices of the Army are described in Technical Manual No. 10-410 of the War Department (1942). A later publication by the Office of the Quartermaster General (1943) contains condensed information on the organization, operation, and administration of the bakery company. Such a company consists ordinarily of five commissioned officers and 163 enlisted men, and is equipped and trained to supply fresh bread for approximately 40,000 men daily.

The bread which is made by Army bakers is distinctive and unlike the usual type of commercial white bread. Although in some training areas in this country much of the bread consumed has been commercial white bread obtained from nearby bakeries, in places where the Army could not avail itself of local facilities, or otherwise has found it desirable to do so, it has established its own bakeries. The type of bread produced in relatively permanent Army bakeries is commonly known as garrison bread and is of two types, a 1½-lb pan loaf and a 10-lb sheet which consists of five 2-lb loaves baked in a single pan. In both of these types of bread the grain is closer and the texture more compact than is usually found in commercial bread, and the crust is thicker and browner in color. The bread baked in field ovens is that used most frequently overseas and in combat areas, and is spoken of as field bread. There are two types of field bread, the most common being that baked in a 10- or 12-lb sheet of five or six 2-lb loaves. The other type is a round loaf weighing 4 lb with an unusually hard and thick crust, with practically no break or shred, the appearance of which has been described by Platt (1924) as similar to "the shell of a turtle." It is a circular loaf about 11 inches in diameter and 4 to 5 inches thick at the

[1] Published with the consent of the Office of the Quartermaster General, Washington, D. C.

highest point. It is a loaf designed to withstand hard usage, shipping in trucks without crushing, and delays of as long as two or three weeks before consumption. Like most Army breads these loaves are subjected to a longer baking time than is the case with the usual commercial loaf of white bread sold in the United States.

The ingredients of Army bread are few in number and the formulas, with the exception of the garrison 1¼-lb bread, may be described as somewhat lean. The dough formulas of the breads examined in the course of the present investigation are provided in Table I and they are typical of the composition of doughs ordinarily used. No yeast foods or bread improvers are employed as a rule, and in the field breads especially, the amounts of sugar and shortening are low. As in commercial bakeries, the use of milk products is customary and Army specifications call for nonfat dry milk solids. There is some variation in the amount of milk that is recommended. The Quartermaster Handbook TM 10-415 states that "in tropical climates or when high humidity exists, the decision as to the quantity of milk used or whether milk be used will be that of the officer in charge." The Handbook points out, however, that "milk in bread is so important from a nutritional standpoint that its use should be encouraged whenever possible."

Army specifications require that the flour shall be a straight grade hard wheat flour of good baking quality. Beginning in 1942 it was required that all flour purchased by the Army for the baking of bread should be enriched in accordance with the standards then promulgated by the Food and Drug Administration. Beginning in the fall of 1943 when the new standards for enriched flour were put into effect, the Army likewise received flour which met these amended specifications.

The amount of bread eaten by soldiers in the United States is considered to be from about 4 to 6 oz daily. Reports about consumption overseas indicate that the amount is increased considerably and the average daily intake is said to be about 8 oz or even more in some theaters of operation.

Because of the importance of bread in the diet of the soldier, it was considered desirable to obtain some data about the actual composition of Army bread from which its nutritive value could be computed. Further, because of the longer time of baking field breads, it was considered necessary to obtain some actual evidence of the degree of retention of thiamine in the baking of these breads. The American Institute of Baking was requested to obtain this information by the Committee on Food Composition of the Food and Nutrition Board of the National Research Council. The work reported herein provides data about the composition from the nutritional point of view of the four common types of Army bread, both at the present level and at the former level

of enrichment, and it also provides some data on the amount of thiamine lost in baking. This work was made possible through the active collaboration of the Quartermaster Corps, United States Army, both at the School for Advanced Baking Instruction which was conducted in Chicago at the American Institute of Baking and at the Cooks and Bakers School, Fort Sheridan, Illinois.

Experimental

Retention of Thiamine. The breads examined were produced according to Army routine at Fort Sheridan. The formulas used in preparing the dough for each type of bread are provided in Table I.

TABLE I

DOUGH FORMULAS

Ingredients	Type of bread			
	Garrison 1¼-lb	Garrison 10-lb sheet	Field 10-lb sheet	Field 4-lb round
	%	%	%	%
Flour (enriched)	100	100	100	100
Water (absorption)	63	62	54	50
Yeast, compressed	2	1.5	—	—
Yeast, dehydrated	—	—	1	0.5
Salt	2.5	2.5	2	2
Sugar	3	1	3	2
Nonfat dry milk solids	6	2	2	2
Shortening	5	3	2	1

The straight dough method was used throughout. The so-called garrison loaves were baked in both reel and hearth type ovens heated by gas. The so-called field loaves were baked out of doors in either the old field or new field ovens. The new field oven officially is designated as the M-1942 Army Field Bake Oven. The old type equipment is designated as Field Oven No. 1. The latter is supplied with the Wynne Oil Burner which burns kerosene. The M-1942 Army Field Bake Oven employs the M-1937 fire unit which burns gasoline. Essential information regarding the oven times and temperatures for these experimental loaves is provided in Table II. The long baking time to which field bread is subjected is noteworthy.

When the doughs were ready for make-up a number of pans of each type of bread were specially scaled at weights enumerated in Table III. The dough for the garrison 1¼-lb loaves was placed in four-strap pans; the dough for the garrison sheet loaves was spaced five loaves in a single large pan and allowed to proof and bake together. The field sheet loaves were handled in a similar manner. The dough for the 4-lb field bread (round loaves) was placed two loaves on a pan, and these did not

touch one another in proofing and baking. When the pans were ready for the oven, samples were packed in dry ice and brought to the laboratory for thiamine analysis. The remaining pans were baked according to the usual practice and pains were taken to see that all the ovens were normally loaded during the baking. When removed from the oven, samples were taken, air dried, and ground for analysis. In sampling, four loaves of garrison 1¼-lb bread, one entire loaf of 4-lb field bread, and one entire sheet each of garrison sheet and field sheet bread were taken. Thiamine determinations on both doughs and breads were made by the collaborative thiochrome method of Hennessy (1942).

The results of the determinations are provided in Table III. No differences in the bake-out loss of thiamine were observed between

TABLE II

OVEN TIMES AND TEMPERATURES

Type of bread	Type of oven and fuel	Oven temperature	Baking time
		°F	min
Garrison 1¼-lb	Reel (gas)	440	35
	Hearth (gas)	440	35
Garrison 10-lb sheet	Reel (gas)	440	44
	Hearth (gas)	440	44
Field 10-lb sheet	New field (gasoline)	450—Start 360—To finish	80
	Old field (wood)	440—Start 360—To finish	80
Field 4-lb round	New field (gasoline)	440—Start 360—To finish	75
	Old field (wood)	475—Start 360—To finish	80

loaves baked in reel and in hearth ovens. A little less thiamine appears to be lost in baking garrison sheet loaves than in baking garrison 1¼-lb loaves. No doubt this result is owing to the relatively greater amount of crust in the 1¼-lb pan loaf, for, as Schultz, Atkin, and Frey (1942) have shown, the greater part of thiamine destruction occurs in the crust of the bread. With the field breads, however, there was an appreciably greater bake-out loss, probably owing to the longer baking period. Schultz, Atkin, and Frey (1942) have found that the amount of thiamine destroyed is markedly influenced by the length of the time of baking. More data would be desirable to ascertain whether there is any significant difference in bake-out loss of thiamine in bread baked in the two types of field oven, but the data obtained indicate that the losses are less in the new type ovens.

Hoffman, Schweitzer, and Dalby (1940) reported a loss of 5% in baking high B_1 bread, but they based their calculations on the assumption that the loss in the innermost crumb was negligible, and Schultz, Atkin, and Frey (1942) assert that the loss of thiamine in this part of the loaf is not insignificant. Melnick, Mabardie, Bernstein, and Oser (1941) found thiamine losses ranging from 9% to 30%, average 19%, in nine enriched white laboratory baked loaves. Schultz, Atkin, and Frey (1942) found a destruction of 21% in enriched 1-lb white loaves baked in the laboratory, and they reported also that commercial bakery tests agreed with the laboratory results. Brown, Hamm, and Harrison (1943) noted a thiamine loss of 15.5% in the experimental baking of enriched bread. It would thus appear that the retention of thiamine

TABLE III

THIAMINE LOSSES IN BAKING FOUR TYPES OF ARMY BREAD

Type of bread	Oven	Scaling weight	Thiamine		
			In dough	In bread	Bake-out loss
		g	*μg/loaf*	*μg/loaf*	%
Garrison 1¼-lb loaf	Reel	680	1749	1447	17
	Hearth	680	1749	1453	17
Garrison 10-lb sheet	Reel	1060	2720	2331	14
	Hearth	1060	2720	2348	14
Field 10-lb sheet	New field	1060	2949	2368	20
	Old field	1060	2949	2312	22
Field 4-lb round	New field	2040	5696	4618	19
	Old field	2040	5696	4323	24

in the baking of Army bread compares favorably with the retention observed in ordinary commercial practices.

Composition of Army Bread. The bread which was analyzed was taken from loaves which were baked by Army men receiving instruction in bakery operations. The dough formulas and the baking procedures were approximately the same as stated in Tables I and II with the following exceptions: 1.25% and 0.75% dehydrated yeast were used in the field sheet and the 4-lb field doughs respectively, and 0.25% yeast food (Arkady) was used in the garrison 1¼-lb and garrison sheet loaves of the bread baked at former enrichment levels. The garrison breads were baked in a traveling tray, oil-heated oven. The bread was air-dried in the dark and ground in a mortar for analysis in order to avoid loss of riboflavin and contamination with iron. Thiamine was determined by the yeast fermentation method including the sulfite modification described by Schultz, Atkin, and Frey (1942a), niacin by the mi-

crobiological method of Snell and Wright (1941) as modified by Krehl, Strong, and Elvehjem (1943), riboflavin by the microbiological method of Snell and Strong (1939) as modified by Andrews, Boyd, and Terry (1942), iron by the method of Andrews and Felt (1941), calcium by the procedure described by Shohl and Pedley (1922), and phosphorus by the

TABLE IV

PROXIMATE ANALYSES OF ARMY BREAD

Type of bread	Moisture	Protein		Fat		Ash		Carbohydrate (by difference)	
		As consumed	Dry basis	As consumed	Dry basis	As consumed	Dry basis	As consumed	Dry basis
	%	%	%	%	%	%	%	%	%
Garrison 1¼-lb loaf	29.1	10.21	14.56	4.54	6.49	2.41	3.44	53.74	75.51
Garrison 10-lb sheet	33.7	9.47	14.34	3.32	5.03	2.13	3.24	51.38	77.39
Field 10-lb sheet	31.0	9.85	14.30	2.47	3.58	1.84	2.67	54.84	79.45
Field 4-lb round	34.0	9.47	14.36	1.42	2.15	1.75	2.66	53.36	80.83
Flour (as used)	13.27	12.03	—	—	—	0.44	—	—	—

TABLE V

VITAMIN AND MINERAL COMPOSITION OF ARMY BREAD

Type of bread	Moisture	Thiamine	Niacin	Riboflavin	Iron	Calcium	Phosphorus
	%	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*
PRESENT ENRICHMENT LEVELS							
Garrison, 1¼-lb loaf	29.3	1.32	13.7	1.36	11.9	318	490
Garrison, 10-lb sheet	35.1	1.23	12.5	1.08	11.1	182	372
Field, 10-lb sheet	30.6	1.31	13.3	1.22	12.3	172	445
Field, 4-lb round	31.4	1.27	13.2	1.08	13.2	159	386
Flour	13.37	2.32	17.4	1.20	14.5	—	—
Enriched bread minimum standard	—	1.10	10.0	0.70	8.0	—	—
FORMER ENRICHMENT LEVELS							
Garrison, 1¼-lb loaf	29.1	1.16	6.56	—	6.6	363[1]	468
Garrison, 10-lb sheet	33.7	1.05	6.02	—	8.5	209[1]	372
Field, 10-lb sheet	31.0	1.18	6.41	—	7.9	154	427
Field, 4-lb round	34.0	1.15	6.03	—	7.1	159	368
Flour	13.27	2.22	7.25	—	7.2	—	—
Enriched bread minimum standard	—	1.00	4.00	—	4.00	—	—

[1] It is estimated that about 50 mg of calcium in each pound of these loaves can be attributed to the inclusion of 0.25% Arkady yeast food in the formulas. Ordinarily yeast foods are not used in the baking of Army bread.

Truog and Meyer (1929) colorimetric method on wet ashed samples. Determinations of moisture, protein, fat, and ash were made according to the methods of the A.O.A.C.

The results of the average values obtained in the analyses of Army bread are provided in Tables IV and V. These values were obtained on bread from 24 to 38 hours after baking. They show that in general the Army bread, except the garrison sheet loaves, contains somewhat less moisture than ordinary commercial bread of the same age, which averages about 35.5% moisture content. The differences in the formulas are reflected in the results. The bread containing greater amounts of shortening had a higher level of fat. The bread containing the

TABLE VI

RETENTION OF THIAMINE, RIBOFLAVIN, AND NIACIN IN FIELD ROUND LOAF IN STORAGE

Time after baking	Moisture	Thiamine (dry basis)	Niacin (dry basis)	Riboflavin (dry basis)
days	%	*mg/100 g*	*mg/100 g*	*mg/100 g*
PRESENT LEVEL OF ENRICHMENT				
1	31.4	0.41	4.26	0.35
7	25.3	0.42	4.30	0.36
14	19.9	0.40	4.25	0.35
FORMER LEVEL OF ENRICHMENT				
1½	34.0	0.38	2.01	—
3	31.9	0.38	2.02	—
5	29.4	0.38	2.01	—
8	28.6	0.37	2.02	—
16	21.9	0.37	2.00	—

greater amounts of nonfat dry milk solids had higher amounts of protein, calcium, phosphorus, and riboflavin.

The values for the vitamin and mineral content of Army bread have been tabulated according to those observed with bread made with the first level of enrichment and those observed with bread after the amended standards of the Food and Drug Administration, requiring a higher content of thiamine, niacin, and iron and requiring also the inclusion of riboflavin, went into effect October 1, 1943. For comparison the minimum standards for the thiamine, riboflavin, niacin, and iron values of enriched bread are listed. It is evident from the data in the tables that Army bread is well above the minimum requirements for enriched bread.

Effect of Storage. Because the field round loaf is designed to be consumed occasionally after appreciable lengths of time in storage or

transit, a number of loaves of this bread were held at room temperature for varying lengths of time and then analyzed for moisture, thiamine, niacin, and riboflavin.

As shown in Table VI, there is no change in the content of thiamine, niacin, and riboflavin in the field round loaf on standing for as long as 16 days. The only change observed was, of course, a gradual loss of moisture, but the bread at the end of this period was palatable. Although this bread kept well, there were some loaves in which after two weeks beginning mold development was noted in the thin crust at the bottom of one or more of the five docker marks placed in the top of the loaf.

Summary

The characteristics of the four types of white bread baked by the Army, namely garrison 1¼-lb loaves, garrison 10-lb sheet loaves, field 10-lb sheet loaves, and field 4-lb round loaves, are described. The composition of these breads varies, of course, with the nature of the ingredients, which, however, are within rather narrow limits prescribed by Army regulations. All U. S. Army bread is made with the use of enriched flour and the present experiments show that the loss of thiamine in the baking varies from 14 to 24%, depending on the nature of the loaf and the treatment to which it is subjected, a result which compares favorably with those observed in the baking of commercial enriched white bread. The inclusion of nonfat dry milk solids in the formula increases the content of protein, calcium, phosphorus, and riboflavin in the loaf, as would be expected. There is no loss of thiamine, riboflavin, or niacin in the field round loaf after storing at room temperature for as long as two weeks, a fact of significance because this type of loaf is designed to be edible after rather long periods of time. Under all conditions encountered Army bread more than meets the minimum standards for enriched bread.

Literature Cited

Andrews, J. S., Boyd, H. M., and Terry, D. E.
1942 Riboflavin analysis of cereals. Application of the microbiological method. Ind. Eng. Chem. (Anal. Ed.) **14**: 271–274.

—— and Felt, C.
1941 The iron content of cereals. Cereal Chem. **18**: 819–826.

Bell, G.
1882 Notes on bread making, permanent and field ovens, and bake houses. Washington, D. C.

Brown, E. B., Hamm, J. C., and Harrison, H. E.
1943 Comparison of thiamine values by chemical and bioassay methods. J. Biol. Chem. **151**: 153–161.

Hennessy, D. J.
1942 A standard thiochrome assay for the determination of thiamin in cereal products. Cereal Chem. Bull. **2**(2): 25–29. (Amer. Assoc. Cereal Chem.)

Hoffman, C., Schweitzer, T. R., and Dalby, G.
1940 The loss of thiamine in bread on baking and toasting. Cereal Chem. **17**: 737–739.
Krehl, W. A., Strong, F. M., and Elvehjem, C. A.
1943 Determination of nicotinic acid. Modifications in the microbiological method. Ind. Eng. Chem. (Anal. Ed.) **15**: 471–475.
Melnick, D., Mabardie, A., Bernstein, A., and Oser, B. L.
1941 The fate of vitamin B_1 in the production of baked goods. N. W. Miller **208** (5): 17–18.
Platt, W.
1924 Making bread for men at war. Baking Technology **3**: 248–252.
Schultz, A. S., Atkin, L., and Frey, C. N.
1942 The stability of vitamin B_1 in the manufacture of bread. Cereal Chem. **19**: 532–538.
1942a Determination of vitamin B_1 by yeast fermentation method. Improvements related to use of sulfite cleavage and a new fermentometer. Ind. Eng. Chem. (Anal. Ed.) **14**: 35–39.
Shohl, A. T., and Pedley, F. G.
1922 A rapid and accurate method for calcium in urine. J. Biol. Chem. **50**: 537–544.
Snell, E. E., and Strong, F. M.
1939 A microbiological assay for riboflavin. Ind. Eng. Chem. (Anal. Ed.) **11**: 346–350.
—— and Wright, L. D.
1941 A microbiological method for the determination of nicotinic acid. J. Biol. Chem. **139**: 675–686.
Truog, E., and Meyer, A. H.
1929 Improvements in the Denigès colorimetric method for phosphorus and arsenic. Ind. Eng. Chem. (Anal. Ed.) **1**: 136–139.
U. S. Army, Office of the Quartermaster General
1943 Quartermaster handbook TM 10–415. Bakery Company, Washington, D. C.
War Department
1942 Technical manual. TM 10–410. Army Baker, Washington, D. C.

ANALYSIS OF DATA FOR A.A.C.C. CHECK SAMPLE SERVICE. I. PROTEIN AND THIAMINE RESULTS 1943-44 [1]

W. O. S. MEREDITH

Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba

(Received for publication May 19, 1945)

As a service to its members the American Association of Cereal Chemists recently organized a Collaborative Check Sample Service. Mr. W. R. Urban is chairman of the Check Sample Committee and is responsible for the forwarding of the samples and the compilation of the results. At the request of Mr. Urban, the writer undertook statistical analyses of the data obtained in the first year of the service. The results of some of the analyses are reported in this paper.

The most important object of the check sample service is to provide

[1] Paper No. 77 of the Grain Research Laboratory.

collaborating laboratories with means of determining whether their results are consistently high, or low, or erratic; so this matter has been given first attention in analyzing the data for the six samples of flour distributed during the first year. Although 130 laboratories cooperated during the year, all did not determine the same properties, and some of the laboratories did not analyze all samples for the properties in which they were interested. The statistical studies discussed in the following sections have been confined to comparisons between laboratories that analyzed all six samples and to data for protein and thiamine. Data for other determinations will be examined in the near future.

For the benefit of those interested in the statistical procedures, these are given in a separate section in which the statistical terms are also described.

Protein

Of the 130 laboratories cooperating in the tests only 68 reported protein contents for all six samples. Discussion and analyses have been confined to the data for these 68 laboratories, which are identified throughout by the numbers used in the original check test reports. Samples of the following six flours were distributed during the first year:

Sample	Mean Protein, %
1. Enriched hard wheat patent flour	12.04
2. Enriched low protein family flour	10.87
3. Soft wheat cake flour	8.09
4. Fine ground whole wheat flour	14.13
5. Duplicate of sample No. 1	12.07
6. Low grade Red Dog flour	17.92

Differences between Duplicate Determinations. As samples Nos. 1 and 5 were duplicates, an estimate of the average error of duplicate determinations over all laboratories can be obtained from 68 pairs of values. The standard error of a determination made in one laboratory on one sample is 0.15%, which is high by comparison with the value of 0.10% reported by Geddes and Milton (1939). When the three largest differences are rejected, on the grounds that they represent abnormal variation within samples,[2] the standard error is reduced to 0.10%, the value reported by Geddes and Milton. This result is very satisfactory in view of the fact that analyses of samples 1 and 5 were

[2] Abnormal variations can be detected, in a series of differences or errors, by comparing the distribution of the data with that expected on the basis of probability. According to the law of probability, in any series of determinations on the same sample or series of samples, the distribution of the results about the mean follows a definite pattern that can be expressed mathematically. The standard deviation represents the range about the mean in which 68.2% of the values lie, and 99.1% of the values lie within the range about the mean of 2.6 times the standard deviation. On this basis the distribution of the values obtained can be examined in order to determine whether the range is normal. Values that lie outside the normal range can be regarded as in definite error and can thus be discarded as abnormal.

made about 10 months apart and therefore represent a strict test of precision.

Differences between Laboratory Means over Six Samples. All collaborators will be interested in knowing how the mean value for their own laboratory compares with the general mean over all laboratories, which is 12.52%. The data can best be considered by listing the differences between the means for each laboratory and the general mean. These differences, together with laboratory numbers, are given in Table I. The laboratories are listed in order of decreasing negative difference by columns to the middle of column 4, where

TABLE I

DIFFERENCES BETWEEN LABORATORY MEAN FOR PROTEIN CONTENTS OF SIX SAMPLES AND GENERAL MEAN FOR ALL LABORATORIES

(Laboratories are identified by the first number in each column)

Range of difference from general mean													
−.90 to −.12		−.10 to −.07		−.06 to −.04		−.03 to +.03		+.04 to +.06		+.07 to +.10		+.12 to +.44	
Lab. No.	%	*Lab. No.*	%	*Lab. No.*	%	*Lab. No.*	%	*Lab. No.*	%	*Lab. No.*	%	*Lab. No.*	%
34	−.90 [1]	6	−.10	17	−.06	98	−.03	105	+.04	97	+.07	95	+.12
12	−.22	110	−.09	56	−.06	8	−.03	1	+.05	72	+.07	81	+.12
85	−.17	112	−.09	89	−.05	52	−.03	55	+.05	78	+.08	23	+.14
48	−.14	11	−.09	24	−.04	51	−.03	29	+.05	68	+.09	108	+.17
74	−.14	38	−.08	33	−.04	58	−.02	43	+.06	31	+.09	61	+.32
50	−.12	75	−.08	39	−.04	76	−.02			63	+.09	70	+.44
		26	−.08	106	−.04	83	−.02			27	+.10		
		54	−.08	32	−.04	100	−.01			42	+.10		
		64	−.07	22	−.04	94	−.01						
		91	−.07	82	−.04	49	0.00						
				104	−.04	13	0.00						
				16	−.04	36	0.00						
						86	0.00						
						3	+.01						
						14	+.01						
						62	+.01						
						69	+.01						
						96	+.02						
						46	+.03						
						47	+.03						
						87	+.03						

[1] Value for Sample No. 6 for this laboratory removed and estimated value inserted before any other calculations were made.

the difference is zero, and thereafter in order of increasing positive difference.

The 21 laboratories in the center column are those having means within ±0.03% of the general mean, which is beyond criticism. In the adjacent columns (3 and 5) are the additional 17 laboratories with means within ±0.06% of the general mean, which is good.

The remaining columns require further consideration. The first and last columns contain the 12 laboratories with means differing by ±0.12% or more from the general mean. These are classed as unsatisfactory because statistical analyses show that a difference of

±0.12% from the general mean is significant at the 5% level.[3] These 12 laboratories therefore obtained mean values for six samples which are significantly different from the result obtained by all laboratories as a group, and are therefore considered to be in error.

Subsequent examination of the data showed that the standard error of a single sample, thus also the necessary difference, was abnormally high and that the "normal" necessary difference was 0.07% protein. On this latter basis, the 18 laboratories in the second and second-last columns, which report differences of from ±0.07 to ±0.11%, must be classed as questionable.

Accordingly, of the 68 laboratories, 38 reported mean values over the six samples that are satisfactory, 18 reported results that are of questionable accuracy, and the remaining 12 reported results that are classed as definitely erroneous.

Differences between Laboratory Means for Single Samples. A statistical procedure (see footnote 2) can also be used to examine the laboratory means for each individual sample. These results will be considered briefly. The standard errors for the six samples are 0.18, 0.14, 0.19, 0.12, 0.12, and 0.23% respectively. The result for sample 6 may be higher because of a greater sampling error for Red Dog flour, or because of greater analytical errors for a sample of high protein content, 17.9%. If sample 6 is left out of consideration, the pooled value for the remaining five samples is 0.15%. This is higher than the values of 0.11 and 0.10% reported by Hildebrand and Koehn (1944) and Davis and Wise (1933) for interlaboratory errors. Further examination shows that the distributions of values for samples 1, 2, 3, and 5 are abnormal. After eliminating the 16 values causing the abnormality, the pooled standard error of a single determination for samples 1 to 5 is 0.10%, which agrees with the figure given by Hildebrand and Koehn. The standard error for sample No. 6 is significantly larger than those obtained for the other five samples.

Variability within Laboratories. Differences between laboratory means over six samples do not give a complete account of intralaboratory errors. Thus two laboratories might have mean differences of zero, yet one might have had zero for each of the six samples and the other might have had +0.1 for three samples and −0.1 for the other three. The consistency of the laboratories can be compared by listing the standard errors of the mean difference from the general mean for each laboratory, and these are given in decreasing order in Table II. The mean standard error within laboratories was 0.15% and the range was from 0.03 to 2.07%. Statistical analyses showed

[3] The significant difference was calculated from the standard error of a single determination, which was 0.15%.

that there were significant differences between the standard errors and that the 18 values listed in the first column of Table II can be considered abnormal. When these are removed, the mean standard error of the remainder is 0.09%. This compares quite well with the

TABLE II

STANDARD ERRORS FOR EACH LABORATORY OF DIFFERENCE BETWEEN LABORATORY MEAN AND GENERAL MEAN
(Laboratories are identified by first number in each column)

Range of standard errors of differences							
2.07 to 0.13		0.12 to 0.10		0.09 to 0.07		0.06 to 0.03	
Lab. No.	%	*Lab. No.*	%	*Lab. No.*	%	*Lab. No.*	%
34	2.07	11	.12	6	.09	36	.06
12	.45	43	.12	13	.09	52	.06
108	.42	55	.12	16	.09	76	.06
70	.38	62	.12	46	.09	91	.06
64	.31	85	.12	61	.09	94	.06
68	.26	89	.12	3	.08	104	.06
31	.25	69	.11	33	.08	17	.05
23	.23	87	.11	39	.08	42	.05
1	.20	112	.11	63	.08	100	.05
54	.19	8	.10	72	.08	105	.04
24	.18	22	.10	74	.08	47	.03
29	.16	27	.10	86	.08	49	.03
32	.16	38	.10	26	.07		
81	.16	48	.10	50	.07		
110	.16	75	.10	58	.07		
14	.14	82	.10	78	.07		
56	.14	95	.10	83	.07		
51	.13	96	.10	98	.07		
		97	.10				
		106	.10				

figures given by Davis and Wise (1933), who obtained a preliminary value of 0.09% and a corrected value of 0.08%. However, as these writers dealt with a much larger number of samples, direct comparisons are hardly permissible.

The way in which this additional check brings to light differences between the accuracy of different laboratories is illustrated in Table III. Six pairs of laboratories are listed together with laboratory No. 49, which has the best record of all 68 laboratories. In each pair, with the exception of the last, the two laboratories have essentially the same mean error over all six samples (second-last column), but the results for the first laboratory in each pair are more variable and the standard error is therefore higher (last column).

One other point is worth noting. The individual types of errors are additive and a laboratory may have a high total error because its results are consistently high or low. For example, laboratory 61 reported results that were consistently high. On the other hand, a

laboratory may have a high total error because its results are variable, for example, laboratory No. 108. Laboratories of the first class should have much less difficulty in tracing the cause of errors than laboratories of the second class.

TABLE III

DATA FOR INDIVIDUAL DIFFERENCES BETWEEN LABORATORY AND MEAN VALUES ILLUSTRATING CONSISTENT AND VARIABLE ERRORS

Laboratory	Sample number						Mean	Standard error
	1	2	3	4	5	6		
	%	%	%	%	%	%	%	%
12	−1.04	+0.10	+0.01	+0.04	+0.02	−0.44	−0.22	0.45
85	−0.06	−0.11	−0.11	−0.08	−0.35	−0.29	−0.17	0.12
64	+0.21	−0.67	+0.01	+0.02	−0.07	+0.08	−0.07	0.31
17	−0.04	−0.10	−0.10	−0.08	−0.07	+0.03	−0.06	0.05
51	+0.12	+0.13	−0.09	−0.03	−0.07	−0.22	−0.03	0.13
52	+0.03	+0.02	−0.12	−0.02	−0.06	+0.02	−0.03	0.06
49	−0.04	−0.01	+0.01	+0.01	−0.03	+0.03	0.00	0.03
14	−0.04	−0.02	−0.03	−0.10	−0.02	+0.28	+0.01	0.14
3	+0.06	−0.04	+0.11	−0.03	−0.11	+0.06	+0.01	0.08
1	−0.04	−0.02	−0.09	+0.07	+0.43	−0.07	+0.06	0.20
55	+0.01	+0.13	−0.09	+0.07	−0.07	+0.23	+0.05	0.12
108	−0.02	−0.02	+1.01	−0.04	−0.11	+0.20	+0.17	0.42
61	+0.26	+0.31	+0.49	+0.32	+0.27	+0.26	+0.32	0.09
Mean	12.04	10.87	8.09	14.13	12.07	17.92	12.52	0.09

Thiamine

Twenty laboratories reported results for all six samples. The data showed a very high degree of variability, and the mean value was 3.30 mg thiamine per lb. The difference between the general mean and the laboratory mean for each laboratory is given in Table IV. It was found that although the mean values for the laboratories ranged from 2.39 [4] to 3.74 mg/lb, the differences between laboratory means could not be considered significant, as the standard error of a single sample was 0.52 mg/lb. The data were examined in several ways in an attempt to derive more concrete information on the extent and location of the variability. With the removal of the results of seven laboratories which showed the greatest variability, the standard error was reduced to 0.30 mg/lb. On this basis, the necessary difference from

[4] Value for sample No. 6 for this laboratory removed and estimated value inserted before any other calculations were made.

TABLE IV

COMPARISONS AMONG LABORATORIES FOR MEAN DIFFERENCE FROM GENERAL MEAN AND STANDARD ERROR OF DIFFERENCE FOR THIAMINE CONTENT IN MG/LB OF SIX SAMPLES OF FLOUR

Laboratory	Mean difference from general mean	Standard error of difference	Laboratory	Mean difference from general mean	Standard error of difference
	mg/lb	*mg/lb*		*mg/lb*	*mg/lb*
89	−0.91 [1]	2.32*	50	0.00	0.27
94	−0.61	1.16*	6	+0.05	0.39
77	−0.35	0.76*	13	+0.11	0.14
24	−0.26	0.62*	75	+0.13	0.25
16	−0.19	0.33	51	+0.14	0.09
110	−0.14	0.39	44	+0.17	0.57*
22	−0.12	0.26	61	+0.17	0.45
57	−0.10	0.15	42	+0.29	0.37
96	−0.06	0.51	54	+0.30	0.74*
100	−0.01	0.08	78	+0.43	0.82*

General mean over all laboratories and samples..... 3.30 mg/lb
Original mean standard error of single sample..... 0.52 mg/lb
Normal mean standard error of single sample after reduction from heterogeneous series..... 0.30 mg/lb

[1] Value for Sample No. 6 for this laboratory removed and estimated value inserted before any other calculations were made.
* Significantly different from normal standard error.

the mean is 0.26 mg/lb and seven laboratories might be considered to have differed from the mean value.

The standard errors within samples, after correction for one abnormal value in each of samples 1, 5, and 6, are shown in Table V. The variation within sample No. 6 is very great. The coefficient of variability is not as great as that for Sample No. 3, a soft cake flour, but, as Hildebrand and Geddes (1943) have shown clearly that the coefficient of variability decreases with increase in thiamine content, it can be concluded that the variation in Sample No. 6 is unduly great. The mean standard deviation for Samples 1 to 5 is 0.21 mg/lb. Only one of the samples, No. 5, has a standard error similar to the value of

TABLE V

MEAN, NORMAL STANDARD DEVIATION, AND COEFFICIENT OF VARIABILITY FOR EACH SAMPLE FOR THIAMINE MG/LB

Sample	Mean thiamine	Degrees of freedom	Normal standard deviation	Coefficient of variability
	mg/lb		*mg/lb*	%
1	2.53	18	0.138	5.5
2	1.90	19	0.215	11.3
3	1.91	19	0.310	16.3
4	2.09	19	0.203	9.7
5	2.18	18	0.104	4.8
6	9.21	18	1.204	13.1

0.12 mg/lb found by Hildebrand and Geddes, and the value of 0.31 mg/lb for Sample No. 3 is quite high.

The variability within laboratories was also examined and the standard error of the mean error for each laboratory is included in Table IV. Seven values, which are marked in Table IV, were significantly greater than the original mean. These were eliminated and a new mean value calculated. This new normal value of 0.30 mg/lb is considerably greater than the value 0.12 mg/lb of Hildebrand and Geddes. There is evidence in Table IV that there is an association of errors in the thiamine determination. It was mentioned earlier that on the basis of the revised error the results of seven laboratories might be considered to have differed from the mean value. It seems more than coincidence that six of the seven laboratories were included in the set of seven that showed very high variability.

Discussion and Recommendations

The determination of protein content of wheat and flour is a routine procedure in all cereal laboratories and a great deal of effort has been made over a long period of time in order to standardize the procedure and to be able to obtain concordant results in different laboratories. This Check Sample Service is undertaken as a continuing effort to examine various analytical procedures and to enable collaborators to take any steps necessary to standardize their technique. The individual laboratories can determine their relative positions with respect to difference from general mean and standard error, or variability, on individual samples in Tables I and II. However, it is worth while to consider the general picture. Though six samples may seem rather few on which to base conclusions, the results of analyses of these samples in 68 laboratories provide a useful body of information.

Twelve laboratories had mean values that were significantly different from the general mean on the basis of the original data, and 18 more might be considered different from the mean on the basis of the revised error. That is, 82% of the laboratories agreed on the mean protein content of the six samples, if values from 12.41 to 12.63% can be considered to be in agreement. If, however, the revised error is used as the criterion of significance, the range is from 12.46 to 12.58% protein, and 56% of the laboratories were in agreement.

Though the "normal" error of duplicate determinations and the "normal" interlaboratory errors are in line with those of previous workers, the original values were abnormal and high in magnitude. A total of 16 samples, involving 12 laboratories, out of 408 were discarded in order to get normal errors between duplicates and within samples. Ten of these twelve laboratories, along with eight others,

showed abnormal variability and were eliminated in calculating the normal standard errors of the differences from the sample means. Thus, the results of 20 laboratories were variable in one form or other. Of these, eight had mean differences from the general mean that were significant on the basis of original error, so that 24 laboratories show definite sources of error. If the revised or "normal" error due to variability within laboratories is used as a criterion for judging whether laboratory means are significantly different from the mean, then 13 more laboratories might be considered as in error, though to a lesser extent than the first group of 24 laboratories. This means that 31 out of 68 showed errors that were within the range of random differences. This may seem to be an unduly critical outlook towards the reproducibility of the protein determination, particularly as the results are for only six samples. Nevertheless, the results represent a considerable body of information, and it cannot be ignored that considerable variability was encountered in the results. There appears to be much yet to be done before the variation between laboratories in protein determinations can be regarded as at a satisfactory low level. The data discussed herein represent only one year's tests, and the variability between laboratories in future tests can be reduced if the cooperating laboratories take active steps to eliminate errors already apparent.

With respect to the thiamine determination, the check test data show that the variations within and between laboratories are considerable. Little can be done about the unsatisfactory conditions between laboratories until the reproducibility within each laboratory is improved. Comparisons between laboratories will then be made more critical and the causes of variations between laboratories might then be elucidated. The effect of storage of samples on thiamine content might well be studied. Samples Nos. 1 and 5 were actually duplicates, but cannot be considered as such since the 8 months' storage between analyses appears to have reduced the thiamine content of the flour. This question has a particular bearing on the keeping and handling of standard reference samples of flour.

The significance of differences between laboratories is best measured over a series of samples and it is for this reason that emphasis should be placed on persuading as many of the cooperating laboratories as possible to analyze a full series of samples for the properties in which they are interested. It is also desirable that the laboratories should submit their duplicate results so that all forms of error can be compared directly without having to draw on previous experience or on part of the study as was done with the protein data.

While it is convenient to make statistical analyses at the end of a series of samples or at the end of fixed periods of time, it is worth

while in the interest of the collaborating laboratories to consider making statistical analyses at the time the results are received. These may well take the form of calculation of the mean and standard error for each sample and the cumulative standard error of difference from the sample mean for each collaborator. It is probable that most, if not all, laboratories do this for their own results, but a uniform procedure would have some advantages. By these means the collaborating laboratories would receive information on their relative standing in time to make any adjustments necessary and to determine on the succeeding samples if the steps taken were adequate.

Statistical Procedures

The statistical procedures used involved: analyses of variance; the calculation of error of analysis within laboratories; mean and standard deviations for samples, means and standard errors of mean differences of laboratories; and the testing of the significance of differences between means and between the standard errors of laboratories.

The data were subjected to analysis of variance and the total variance was split into portions due to: (1) differences between laboratory means, (2) differences between sample means, and (3) differences in the relative placing of the samples in the different laboratories. The last mentioned variance, commonly known as the error due to interaction between samples and laboratories, was used to calculate the standard error of a single sample and the necessary difference between a laboratory mean and the general mean. The latter represents the value at which the variation can be attributed to chance only once in 20 times, and was used for testing the significance of differences between laboratories.

The error of duplicate analyses within laboratories was determined by calculating the error due to the duplicate determinations represented by Samples 1 and 5. The standard error of a single determination was calculated according to the formula

$$\text{Standard error} = \sqrt{\frac{\Sigma(x_1 - x_2)^2}{2(N-1)}}$$

when $x_1 - x_2$ is the difference between duplicates and N is the number of duplicate analyses.

The means were calculated in the usual way by the formula $\Sigma x/N$ and the standard error within samples was calculated by use of the formula

$$\text{Standard error} = \sqrt{\frac{\Sigma(x - \bar{x})^2}{N-1}}$$

where x = the value obtained by an individual laboratory, $\bar{x}$ = the mean value for all laboratories, and N is the number of laboratories.

The distribution of results within samples was tested for normality by comparing the range with the standard error. If distributed normally, 68.2% of the results for a series of analyses on the same sample will fall within the range expressed by the mean plus or minus the standard deviation, and 99.1% of all values will not differ from the mean by more than 2.6 times the standard deviation. For the purposes of this paper any value differing from the mean by 3 times the standard deviation was considered abnormal and rejected and a new standard deviation calculated. This procedure was continued until the distribution of the values was normal.

The variability of each laboratory about its own mean systematic error (standard error within laboratories, called the standard error of the mean difference from the general mean in the section on variability within laboratories) was calculated from the formula

$$\text{Standard error of mean difference} = \sqrt{\frac{\Sigma(x - \bar{x})^2 - \frac{[\Sigma(x - \bar{x})]^2}{N}}{N-1}}$$

where x is the value obtained by the laboratory for a particular sample, $\bar{x}$ is the mean value over all laboratories for that sample, and N is the number of samples analyzed.

This intralaboratory error is that portion of the total variance within a laboratory remaining after corrections for differences between samples and for the difference between the laboratory mean and the general mean have been applied.

The homogeneity of the individual intralaboratory variances was tested according to the method of Snedecor (1938, p. 206) and the highest variances were removed progressively until the series was homogeneous and the normal mean error was then established. It seems worth while to observe that this variance for each laboratory is the contribution of the laboratory to the interaction variance, which arises from the failure of the laboratories to place the samples in exactly the same relative order, and the sum of the individual variances equals the interaction variance.

Summary

The data for protein and thiamine content of six flour samples sent out under the A.A.C.C. check sample service in its first year of operation have been examined to determine the interlaboratory error.

Sixty-eight laboratories analyzed the six samples for protein content, and differences between laboratories in mean value were demonstrated. On the basis of a found standard error of a single sample of 0.15% protein, 82% of the laboratories agreed within ±0.12% of the general mean value of 12.52% protein for the six samples. This standard error was found to be abnormal and the normal standard error was calculated to be 0.09% protein; 56% of the laboratories agreed within ±0.07% of the mean protein value. Results on duplicate determinations were available as one flour was represented by two samples. The original standard error of a single determination was 0.15% protein, but the distribution of differences between duplicates was abnormal and when adjusted the standard error was 0.10% protein. The variability within laboratories was also examined and 18 laboratories showed abnormally high variability. Out of the 68 laboratories, 37 showed errors that were outside the range of random differences.

The data for the thiamine determination, which represent results from 20 laboratories, were extremely variable. The original standard error of a single sample was 0.52 mg thiamine per lb and when adjusted for abnormality it is 0.30 mg/lb.

Recommendations are made regarding the handling of the data obtained from future studies.

Acknowledgments

The interest of Dr. J. A. Anderson, chief chemist of this laboratory, in the study and his advice on the preparation of the manuscript are gratefully acknowledged.

Literature Cited

Davis, C. F., and Wise, M.
1933 A five-year summary of the monthly check sample reports of the Pioneer Section of the A.A.C.C. Cereal Chem. 10: 203–212.

Geddes, W. F., and Milton, N.
1939 The analytical error of the Kjeldahl nitrogen test. Cereal Chem. 16: 393–404.

Hildebrand, F. C., and Geddes, W. F.
1943 The experimental error of the thiochrome method for thiamine assay. Cereal Chem. 20: 714–717.
——, and Koehn, R. C.
1944 Sources of error in the determination of the protein content of bulk wheat. Cereal Chem. 21: 370–374.
Snedecor, G. W.
1938 Statistical methods applied to experiments in agriculture and biology. Collegiate Press, Inc., Ames, Iowa.

REPORT OF THE 1944-45 COMMITTEE ON NIACIN (NICOTINIC ACID) ASSAY

HAROLD K. STEELE, Chairman

The Fleischmann Laboratories, New York, N. Y.

(Received for publication May 14, 1945)

Since the discovery of the biological importance of niacin, many methods for its determination have been proposed. The bread and flour enrichment program gave added impetus to the search for a rapid, reliable method. Microbiological methods (Snell and Wright, 1941; Krehl, Strong, and Elvehjem, 1943; the U.S.P. method, 1943) have come into wide use and are considered by many workers to be more reliable than chemical methods. However, the time required for the microbiological assays makes a more rapid method desirable.

A previous collaborative study conducted by Melnick (1942) showed that, under proper conditions, both chemical and microbiological procedures can be used to determine niacin in flour and bread. It was the hope of your chairman that by combining and modifying the methods of Dann and Handler (1941), Melnick, Oser, and Siegel (1941), Melnick (1942), and Hausman, Rosner, and Cannon (1943), a more satisfactory procedure could be developed. The complete procedure, together with some modifications suggested by the collaborators, is herewith reproduced.

A COLORIMETRIC METHOD FOR THE DETERMINATION OF NIACIN (NICOTINIC ACID)

This determination depends on the measurement of the color produced when nicotinic acid reacts with cyanogen bromide and Metol (methyl-paramido-phenol sulfate).

REAGENTS:

Cyanogen Bromide: 26 ml of cold bromine are added to 500 ml of cold water and placed in an ice bath. To this is added cold 10% NaCN solution, drop by drop, with stirring until the bromine is just decolorized. The end point is approached slowly, and the reaction mixture must be kept cold at all times. When the brown color of the bromine has just disappeared, 5 drops excess of the NaCN solution are added, and the solution made to 1 liter. This gives approximately a 0.5 *N* solution of CNBr. This solution will keep for several weeks if stored in a refrigerator in a brown bottle.

Alternative Method of Preparing Cyanogen Bromide: If desired, the CNBr solution may be prepared from crystalline CNBr. Many workers prefer this method of preparation.

Since the method was typed and distributed to the collaborators, it has been found that a 0.25 *M* solution of CNBr is satisfactory. This more dilute solution is not so unpleasant to use as the 0.5 molar solution.

Metol: Merck and Company's "Photol" has been found satisfactory. A 5% solution (wt./vol.) is prepared fresh each day. If this solution is prepared in an amber or red bottle with the addition of 10–20 mg *d*-isoascorbic acid per 500 ml, it will remain practically colorless for 24 hours. However, it is best to prepare it just before use. (See Note 1.)

Hydrochloric Acid: 12 *N*, specific gravity about 1.18.

Sulfuric Acid: 0.2 *N* and 2.0 *N*.

Phosphoric Acid: 10 ml of 85% orthophosphoric acid diluted to 100 ml with distilled water.

Sodium Hydroxide Solutions: Approximately 40% (10 *N*) and 0.4 *N*.

Phenolphthalein Solution: 1% in 95% ethanol.

Methyl Violet Solution: 0.1% in water.

Lead Nitrate: Finely ground.

Tertiary Potassium Phosphate.

Lloyd's Reagent: Manufactured by Eli Lilly and Company.

Nitrazine Paper: Manufactured by E. R. Squibb and Sons.

Standard Nicotinic Acid Solution: A stock solution containing 100 μg/ml in absolute ethanol is stable for several months. For the color reaction, make a fresh dilution daily of 10 μg/ml in water.

SPECIAL APPARATUS: Photoelectric spectrophotometer or colorimeter. Beckman, Cenco, Coleman, Evelyn, or Pfaltz and Bauer may be used. Graduated 50 ml centrifuge tubes, or small bottles for the centrifuging operations. (The writer uses small bottles, i.e., 2 oz Boston rounds with screw tops having rubber liners.)

PROCEDURE:

A sample of the finely divided material, containing 100 to 500 μg of nicotinic acid, is suspended in 75 ml of 2 *N* H_2SO_4 in a 100 ml volumetric flask, and hydrolyzed in boiling water for one hour with occasional mixing. At the end of this period, the flask is cooled to room temperature and made to volume with distilled water. The contents of the flask are well mixed, and a 25 ml aliquot is transferred to a graduated 50 ml centrifuge tube, and the pH adjusted to 0.5–1.0 with 40% NaOH solution, using methyl violet in a spot plate as an outside indicator. One drop of indicator and one drop of the sample are mixed on the spot plate, and the color compared with that produced when one drop of indicator is mixed with one drop of 0.2 *N* H_2SO_4.

One gram of Lloyd's reagent is then added, and the contents of the tube are well mixed for at least one minute. The tube is centrifuged clear, and the supernatant discarded. The Lloyd's reagent is washed twice by suspending it in 10 ml of 0.2 *N* H_2SO_4, centrifuging, and discarding the supernatant. After the final washing, the tube is inverted and allowed to drain well. Sufficient 0.4 *N* NaOH is then added to each tube to make the total volume 25.5 ml, and the contents mixed for at least one minute.

The tubes are then centrifuged, and the supernatant poured into a clean dry centrifuge tube containing 1.6 g of powdered $Pb(NO_3)_2$, and mixed well by swirling. (It is well to add a drop of phenolphthalein indicator at this point to make sure sufficient $Pb(NO_3)_2$ has been added to give an acid reaction. If the sample is alkaline to the phenolphthalein, this indicates that not enough $Pb(NO_3)_2$ has been added, or that the contents of the tube have not been sufficiently well mixed. If further mixing does not decolorize the indicator, more $Pb(NO_3)_2$ is added with stirring until a slight excess is present.) The tubes are then centrifuged and the supernatant poured into a third clean dry centrifuge tube. One drop of phenolphthalein solution is added, then solid K_3PO_4, until the solution is pink. The pH is adjusted to between 5.0 and 6.0 with 10% H_3PO_4 and K_3PO_4, using nitrazine paper as an outside indicator.

The tubes are centrifuged again and the supernatant decanted carefully into a small flask.

For the color development, three 5-ml aliquots of each solution are put into small flasks or test tubes. (Since the color reaction is sensitive to light, red glassware is used, or the solutions are placed in a dark cabinet immediately after the addition of the Metol.) These are marked "a," "b," and "c." 12 ml of water is added to

aliquot "a," and 1 ml of water to aliquot "b." 1 ml of standard nicotinic acid solution, containing 10 μg of nicotinic acid, is added to aliquot "c" from a 10 ml semimicro burette. 1 ml of CNBr reagent is then added to aliquots "b" and "c," and after 15 minutes, 10 ml of the Metol reagent is added. The samples are kept in the dark for one hour, after which time the color is measured in the photoelectric spectrophotometer at 410 mμ. (See notes.)

A reagent blank, containing 6 ml of water, 1 ml CNBr solution, and 10 ml of Metol solution, is conducted along with the samples.

The photometric density, $\log_{10}(I_0/I)$, of the reagent blank and aliquots "a," "b," and "c" is measured at 410 mμ against distilled water in the comparison tube of the photometer. If the instrument measures only percent transmission, photometric densities can be calculated by the formula $d = 2 - \log g$, where d = photometric density, and g = the galvanometer reading in percent transmission. The instrument is adjusted to give a reading of zero density or 100% transmission with distilled water in the comparison cell before reading the sample solutions.

The photometric density of aliquot "a" is due to the residual color of the sample solution. This value, plus that of the reagent blank, is subtracted from the density values of aliquots "b" and "c." The difference, "b" − ("a" + reagent blank), = color due to reacted nicotinic acid in the sample. "c" − ("a" + reagent blank) = color due to the nicotinic acid in the sample, plus the 10 μg added to this aliquot. The difference, "c" − "b", = color developed from 10 μg of pure nicotinic acid, and this value is used to calculate the amount of nicotinic acid in the sample.

For example: if the photometric density of

reagent blank = 0.015,
"a" = 0.005,
"b" = 0.167,
"c" = 0.453,

then "b" − ("a" + reagent blank) = 0.147, "c" − ("a" + reagent blank) = 0.433, and "c" − "b" = 0.286 = the photometric density due to 10 μg of nicotinic acid. Then $\frac{0.286}{10} = 0.0286$ = the color increment per microgram of nicotinic acid, and $\frac{0.147}{0.0286} = 5.14$ μg of nicotinic acid in aliquot "b." This value, divided by the weight in grams of the sample in aliquot "b" = micrograms of nicotinic acid per gram of original sample.

NOTES:

1. Some samples of Metol become colored very quickly, due presumably to photo oxidation. The Metol manufactured by Merck and Company, and sold under the trade name of "Photol" has been found satisfactory.

   The addition of a small amount of *d*-isoascorbic acid has been found to stabilize the Metol solution. The increase in photometric density of a blank solution was only 0.036 after standing 24 hours in a red glass flask.
2. Reaction of Nicotinic Acid with CNBr: Contrary to the findings of Bandier and others, it is not necessary to heat the solution to 80° C for the reaction of nicotinic acid with CNBr when Metol is used as the amine. When CNBr reacts nicotinic acid at room temperature, the color produced after the addition of Metol increases with the time allowed for the CNBr reaction up to 15 minutes, after which there is no significant increase in color.
3. Metol has been selected for use in this method because of the greater stability of the color as compared to the color produced by aniline or p-aminoacetophenone. It is also claimed that Metol has a greater specificity for niacin than other amines which have been used in this reaction. The intensity of color produced per μg of nicotinic acid is as great when Metol is used as is obtained by the use of aniline. The use of Metol also makes it unnecessary to make the color readings at exactly the same time after the addition of the reagents.
4. pH of Sample for Color Development: The intensity of the color produced per μg of nicotinic acid depends on the pH of the test solution at the time the CNBr is added. Good results were obtained at pH values between 5.0 and 6.1, with the optimum at pH 5.8. The final pH of all solutions tested was approximately 2.1.
5. Use of Lloyd's Reagent: Most methods recommend the use of 2.5 g of this material. Following a suggestion of Dr. H. P. Sarett, 1 g of Lloyd's reagent was tried with

good results. This amount is, therefore, recommended for the absorption of nicotinic acid from the hydrolyzed sample of solution.

6. Since the intensity of color developed per μg of nicotinic acid depends upon so many factors, it is necessary to use the increment method for evaluation. The writer believes more precise results will be obtained by the use of 1 ml of a solution containing 10 μg/ml than by 0.1 ml of a solution containing 100 μg of nicotinic acid per ml.
7. 5 gram samples are recommended for the hydrolysis in the hope of obtaining a more representative sample than can be obtained by the use of 1-g samples. According to Dr. J. S. Andrews (personal communication), the use of a fairly large sample is especially recommended for enriched flour. The size of the aliquot given in the method has been found satisfactory for whole wheat flour when the color is measured with the Beckman spectrophotometer. For other instruments and other samples, larger or smaller aliquots may give better results. If possible, the amount of the sample and aliquot should be selected so that the photometric density of aliquot "b" will be 0.150, or greater. This may not be possible when working with unenriched white flour. If samples larger than 5 g in 100 ml are used, the results obtained will be low. This may be due to incomplete extraction, as has been found for riboflavin.

   If desired, the hydrolysis can be carried out with 2 *N* to 4 *N* HCl instead of H_2SO_4. However, the color of the blank will be higher if HCl is used.
8. The wavelength chosen for the color measurement is 410 mμ. This wavelength was selected because at this point in the spectrum the color values of the blanks were lower than at any point between 400 mμ and 420 mμ compared to the color values of the sample. However, the color response per μg of nicotinic acid is linear over a range of wavelength between 400 mμ and 420 mμ.

Preliminary assays in the author's laboratory indicated that this method would give reproducible results somewhat lower than, but of the same order of magnitude as, the U.S.P. microbiological method (1943).

Results of Collaborative Studies

The above procedure, together with three samples,[1] was submitted to the 12 members of the Committee for collaborative study. The collaborators were requested to assay these samples by the proposed method and by any other method or methods currently in use in their laboratories. Ten of the 12 collaborators responded, and the results they submitted are recorded in Table I.

The mean results obtained by the proposed colorimetric method were lower than those obtained by the microbiological methods, but they agree very well with the results obtained by the same laboratories with the chemical methods currently in use in those laboratories. The reproducibility of the results in any one laboratory was also good. The range of results between laboratories, however, is too great for the proposed method to be recommended to the Association for a control method. It should be noted, however, that if one or two especially low or high results could be eliminated from the results on

[1] The samples were prepared as follows:
Sample No. 1 was an unenriched white flour.
Sample No. 2 was this same flour enriched with a commercial enrichment mixture in sufficient amount to increase the niacin content by 14.04 μg/g. (This enriching powder was kindly supplied by Dr. Arnold of the Winthrop Chemical Company, Rensselaer, New York.)
Sample No. 3 was a bread made from sample No. 2.

each sample, the others fall within a satisfactory range, without materially changing the average.

The recovery of added niacin was satisfactory by both chemical and microbiological methods, if the calculated potencies of samples Nos. 2 and 3 were based on the potency determined on sample No. 1 by that method.[2] If the determined instead of the calculated potencies of the enriched flour are used, the calculated potency of the bread is almost exactly the determined value.

It is worthy of note that most laboratories that reported low values by the collaborative procedure also reported low values by other methods. This could be explained on the basis of a high potency

TABLE I

NIACIN ASSAY VALUES OBTAINED BY THE VARIOUS COLLABORATORS

Collaborator	Sample No. 1			Sample No. 2			Sample No. 3		
	Collaborative method	Other chemical method	Microbiological method	Collaborative method	Other chemical method	Microbiological method	Collaborative method	Other chemical method	Microbiological method
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
1	8.5	8.8	—	19.0	19.1	—	19.2	19.5	—
2	7.8	7.5	—	15.0	15.0	—	16.8	17.3	—
3	8.9	12.4	12.0	22.6	24.0	23.8	23.5	24.2	23.2
4	7.4	—	—	18.7	—	—	18.1	—	—
5	—	—	11.2	—	—	25.7	—	—	28.0
6	—	—	—	—	—	26.5	—	—	28.4
7	8.7	—	11.2	22.8	—	24.7	28.7	—	27.5
8	9.5	11.2	10.7	21.2	24.7	24.0	19.7	25.6	26.7
9	15.5	—	13.1	26.4	—	25.8	27.4	—	28.4
10	8.8	—	11.1	22.6	—	24.9	21.7	—	23.3
Maximum	15.5	12.4	13.1	26.4	24.7	26.5	28.7	25.6	28.4
Minimum	7.4	7.5	11.1	15.0	15.0	23.8	16.8	17.3	23.2
Mean	9.4	10.0	11.6	21.0	20.7	25.1	21.9	21.7	26.5

standard solution. One of the collaborators suggested that low results could be due to incomplete elution of niacin from Lloyd's reagent, since this collaborator had encountered some batches of this material which required elution with hot 0.5 *N* NaOH to remove adsorbed niacin completely.

Another possibility is that these discrepancies are caused by the presence in cereals of some unknown material which, under certain conditions, reacts like niacin. The presence of some such material in

[2] The calculated potencies were obtained as follows:

For the recovery calculation, the average potency of sample No. 1, as determined by each method, was assumed to be correct. The known enrichment of sample No. 2 was 14.04 μg/g. Then the determined value for sample No. 1 + 14.04 = calculated potency of enriched flour.

For the bread: 18,466 g enriched flour and 369 g yeast produced 19,250 g air dry bread. Hence

$$\mu g \text{ niacin in 1 g bread} = \frac{\mu g \text{ niacin in enriched flour} + \mu g \text{ niacin in yeast}}{19{,}250}.$$

cereals was first reported by Kodicek (1940), and later by Melnick (1942). If this material were less completely adsorbed upon, or eluted from, some batches of Lloyd's reagent than niacin, these and other differences in assay values could be explained.

This condition should be investigated rather thoroughly, because if this variation in Lloyd's reagent is common, each new lot should be tested before it is used for niacin assay.

It is recommended that work on the chemical determination of niacin be continued.

Comments

The most important by-product of a collaborative study is the comments of the collaborators.

Collaborator No. 1 questioned the necessity of using such a strong cyanogen bromide solution. Subsequent work indicates that the point is well taken, and that half the specified strength is satisfactory.

This collaborator also recommended the use of potassium dihydrogen phosphate solution as a buffer as is done in the Dann and Handler (1941) method, and questions the necessity of the increment method of evaluation. This procedure simplifies the method materially, but your chairman has not found that the color response is sufficiently constant from sample to sample to discard the increment method.

Work with standard solutions at various pH values indicates that more reproducible results were possible if the initial pH of the sample solutions was between 5 and 6 at the time of addition of cyanogen bromide. The use of potassium dihydrogen phosphate may lower the pH too much for consistent results. More work should be done along this line. It should be possible to use solid potassium dihydrogen phosphate or a fairly concentrated solution of this salt in place of the 10% phosphoric acid solution for adjusting the final pH of the sample solutions.

Collaborator No. 2 emphasized the necessity of using a good quality of Metol. Some lots of this material become colored even when *d*-isoascorbic acid is used as an antioxidant.

Collaborator No. 3, like collaborator No. 1, preferred to use commercial cyanogen bromide crystals instead of bromine and sodium cyanide for the preparation of the cyanogen bromide solution.

Aniline is preferred by this collaborator as the color-producing amine. This collaborator called the attention of the writer to the lack of a correction for the liquid occluded on the Lloyd's reagent.

Collaborator No. 4 reported the formation of a precipitate in some

of the solutions during color development. The writer is not sure what caused this in the case of collaborator No. 4, but a precipitate will form if the lead is not completely removed.

Collaborator No. 8 obtained better checks if the hydrolysate was centrifuged, and aliquots taken from the supernatant, and the first pH adjustment made with the glass electrode.

Summary

A tentative chemical procedure, based on the color produced when niacin is reacted with cyanogen bromide and Metol, for the determination of niacin in flour and bread was subjected to collaborative study. Results from 10 laboratories indicated that this method yielded reproducible results which were in good agreement with those obtained by other chemical procedures in use in the same laboratories, but the agreement between different laboratories was not satisfactory.

Acknowledgments

The author wishes to express his gratitude to Mrs. J. M. Buckley and Miss Connie Nott for their assistance in preparing this program, and to the following collaborators who participated in this study: G. Worthen Agee, Borrow-Agee Laboratories, Memphis, Tennessee; J. S. Andrews, General Mills, Minneapolis, Minnesota; L. Atkin, and Adrienne Murset, Fleischmann Laboratories, New York, New York; Sadie Brenner, Chicago Quartermaster Depot, Chicago, Illinois; E. B. Brown, Anheuser-Busch, Inc., St. Louis, Missouri; Howard J. Cannon, Laboratory of Vitamin Technology, Chicago, Illinois; W. J. Dann, Duke University, Durham, North Carolina; C. A. Elvehjem, University of Wisconsin, Madison, Wisconsin; L. W. Hass, The W. E. Long Company, Chicago, Illinois; M. C. Kik, University of Arkansas, Fayetteville, Arkansas; D. Melnick, Food Research Laboratories, Long Island City, New York; H. P. Sarett, Oregon State College, Corvallis, Oregon.

Literature Cited

Dann, W. J., and Handler, P.
1941 The quantitative estimation of nicotinic acid in animal tissues. J. Biol. Chem. **140**: 201–213.

Hausman, E., Rosner, L., and Cannon, H. J.
1943 The preparation and decolorization of cereal extracts for nicotinic acid determination. Cereal Chem. **20**: 82–86.

Kodicek, E.
1940 Estimation of nicotinic acid in animal tissues, blood and certain foodstuffs. Biochem. J. **34**: 712–723.

Krehl, W. A., Strong, F. M., and Elvehjem, C. A.
1943 Determination of nicotinic acid. Ind. Eng. Chem. (Anal. Ed.) **15**: 471–475.

Melnick, D.
1942 Collaborative study of the applicability of microbiological and chemical methods to the determination of niacin (nicotinic acid) in cereal products. Cereal Chem. **19**: 553–567.

——, Oser, B. L., and Siegel, L.
1941 Chemical determination of nicotinic acid content of flour and bread. Ind. Eng. Chem. (Anal. Ed.) **13**: 879–883.

Snell, E. E., and Wright, L. D.
1941 A microbiological method for the determination of nicotinic acid. J. Biol. Chem. **139**: 675–686.

First U. S. Pharmacopoeia XII, Bound Supplement. 1943.

REPORT OF THE 1944–45 COMMITTEE ON RIBOFLAVIN ASSAY

AARON ARNOLD, Chairman

Winthrop Chemical Company, Inc., Research Laboratories, Rensselaer, New York

(Received for publication April 26, 1945)

This Association has taken the initiative in attempting to establish the value of the fluorometric procedure for the estimation of riboflavin. Under the guidance of Andrews (1943, 1943a, 1944) a rapid, simple procedure has been developed. The procedure depends upon the extraction of the vitamin with dilute acid, filtration, and treatment of the filtrate with permanganate and hydrogen peroxide preparatory to measurement of the fluorescence of the extracted riboflavin. Evaluation of the vitamin content of the extract is based upon an internal standard. This procedure does not utilize Florisil adsorption and elution recommended in earlier studies (Andrews, 1943a), since its use was shown to result in wide variation in the results from different laboratories (Andrews, 1944).

The collaborative program was planned to compare the results of three assay methods as applied to flour containing added riboflavin and to bread baked from that flour, to compare the results of assays on 1.5 and 3.0 g samples using the method supplied, and to compare, somewhat indirectly, two methods of extraction of the vitamin from the starting material. The first objective was achieved by requesting the collaborators to assay the flour and bread by the method supplied and by the fluorometric method in use in their laboratory, as well as microbiologically where possible. The second objective was to determine whether greater reproducibility without loss in precision could be obtained by increasing the size of the sample analyzed. The third objective was achieved by comparing the results of the group of collaborators which chose to extract the samples by autoclaving with those of the group which extracted at the temperature of boiling water.

The method supplied was the same, except for slight simplification, as that used in the previous Association study. The collaborators were requested to indicate the make of instrument used in their work.

The response to the request for collaboration was surprisingly good, in view of the limited help now available, and the results of 27 laboratories are herewith presented.

The proposed procedure is given below:

FLUOROMETRIC PROCEDURE FOR RIBOFLAVIN ASSAY

REAGENTS:

(1) Sulfuric acid solution, 0.1 *N*

(2) Sodium acetate solution, 2.5 M (340 g $C_2H_3O_2Na \cdot 3H_2O$ per 1,000 ml)

(3) Potassium permanganate solution, 4% (freshly prepared)
(4) Hydrogen peroxide solution, 3%
(5) Acetone (low fluorescent blank)
(6) Sodium fluorescein stock solution, 10 mg/1,000 ml (10 μg/ml)
(7) Sodium fluorescein dilute solution, 0.1 μg/ml
(8) Riboflavin standard solution, 1 μg/ml
(9) Sodium hydrosulfite, powder

APPARATUS:

(1) Fluorophotometer
(2) Cylinders, glass stoppered, graduated to 100 ml
(3) Autoclave or water bath
(4) Erlenmeyer flasks, 250 ml
(5) Volumetric flasks, 100 ml

PROCEDURE:

The sample [1] of the flour or bread is uniformly suspended in approximately 75 ml of 0.1 *N* sulfuric acid. The suspension is then autoclaved for 15 minutes at 15 lb pressure or heated in a boiling water bath for 45 minutes [2] with intermittent shaking. After cooling to room temperature, the suspension is adjusted to pH 4.3 with 2.5 M sodium acetate (approximately 5.0 ml). The suspension is diluted to 100 ml with distilled water in a volumetric flask, well shaken, and filtered through Whatman No. 1 or No. 40 filter paper. The first 10 or 15 ml of filtrate may be discarded or allowed to drain directly back into the main portion of the suspension.

Treat 60 ml of filtrate with 2 ml of 4% potassium permanganate solution.[3] After 3 minutes, add enough 3% hydrogen peroxide solution to discharge the permanganate color (should not require more than 2 ml). Discharge the froth with a few drops of acetone [4] and dilute to 65 ml with distilled water. Filter and mix thoroughly.

Pipette two 15 ml portions of the filtrate into two 25 ml cylinders or flasks. To one add 1 ml of distilled water. To the second add 1 ml of riboflavin standard solution. Mix contents of each.

Measure the fluorescence of the sample (reading A), of the sample which contains 1 μg of added riboflavin (reading B), and of the blank (reading C) of the latter solution obtained by the addition of a standardized amount of sodium hydrosulfite powder (not more than 10 to 20 mg).

Calculation:

$$\mu\text{g riboflavin per gram sample} = \frac{\text{fluorescence due to sample}}{\text{fluorescence due to added riboflavin}} \times \text{riboflavin increment} \times \frac{\text{dilution factor}}{\text{wt. sample}}$$

OR

$$\mu\text{g riboflavin per gram sample} = \frac{A - C}{B - A} \times \frac{7.22}{\text{wt. sample}}$$

where $A - C$ = fluorescence due to sample
$B - A$ = fluorescence due to added riboflavin
1 μg = riboflavin increment
7.22 = dilution factor $\left(\frac{1}{15} \times \frac{65}{60} \times 100\right)$

Two samples were submitted to the participating laboratories: (1) a flour sample [5] supplemented with riboflavin to a little over half

[1] The sample must be finely ground for good extraction.
[2] If a boiling water bath is used, the sample may be placed directly into a 100 ml volumetric flask. Samples to be autoclaved must be placed in larger flasks (250 ml Erlenmeyers, for example).
[3] Some cereal products will require more permanganate than the amount specified here. This may be accomplished with the use of a stronger permanganate solution (7%), so that the volumes conform with those given here, or with the use of the 4% permanganate solution which will necessitate slightly greater dilution of the 60 ml aliquot.
[4] The froth, when present, should be allowed to subside for a minute or two before the acetone addition. One collaborator observed that a drop of caprylic alcohol serves equally well.
[5] The writer is indebted to Mr. H. K. Steele, Fleischmann Laboratories, who supplied the samples for this study.

the amount required legally (1.2 mg/lb), and (2) an air-dried, ground bread baked from a portion of the aforementioned flour.

Of the 27 collaborators, 20 submitted results by a fluorometric method in use in their own laboratories which differed from the proposed procedure in varying degrees. In addition, 11 collaborators submitted the results of microbiological assay.

Discussion of Results

FLOUR SAMPLE

In Table I is given the analysis of results submitted by the collaborators on the analysis of the flour sample that contained approximately 1.56 μg riboflavin per gram.[6]

TABLE I

COMPARISON OF RESULTS OBTAINED BY THE COLLABORATIVE STUDY ON THE RIBOFLAVIN CONTENT OF THE FLOUR [1] SAMPLE

Procedure	Results of all collaborators			Results of collaborators within ±20% of 1.56 μg/g [2]	
	Range assay results	No. collab.	Mean ±S.E. of assay results	No. collab.	Mean ±S.E. of assay results
	μg/g		μg/g		μg/g
Proposed method					
1.5 g sample					
autoclave	1.34–1.82	17	1.53±0.04	17	1.53±0.04
hot water	1.14–1.60	8	1.42±0.06	6	1.49±0.03
3.0 g sample					
autoclave	1.27–1.93	17	1.47±0.04	16	1.45±0.02
hot water	1.18–1.55	8	1.39±0.05	6	1.46±0.04
Collab. own methods					
autoclave	1.32–1.92	13	1.56±0.04	12	1.53±0.04
hot water	1.15–1.67	7	1.50±0.07	6	1.56±0.05
Microbiological method	1.26–2.51	11	1.71±0.10	8	1.55±0.06

[1] Calculated riboflavin content = 1.56 μg/g.
[2] 1.25 to 1.87 μg/g.

Range of Values. Most of the collaborators reported results within ±20% of the calculated riboflavin content of the sample. Of the 81 results broken down into the seven categories given in Table I, there were five results below 80% and an equal number above 120%. On the assumption that such results are due to reasons outside the limitations of the method, they should not be permitted to obscure the results obtained by the majority of the laboratories. Hence the averages in

[6] 20.4 mg of riboflavin supplied by 100 lb of flour (0.45 μg/g) and 50.58 mg of riboflavin added to give a sample calculated to contain 1.56 μg/g.

the last column of Table I are based on the results that are within ±20% of the calculated riboflavin content of the sample. The mean of the results in this range are thus in agreement with each other and with the expected value.

It is clear from these results that collaborators can obtain agreement in assaying flour samples, using the method proposed, their own fluorometric procedures, or the microbiological procedure.

Influence of the Size of Sample. While the mean of the results on the 3.0 g sample by the proposed procedure in the 80 to 120% group

TABLE II

COMPARISON OF RESULTS OBTAINED BY THE COLLABORATIVE STUDY ON THE RIBOFLAVIN CONTENT OF THE BREAD [1] SAMPLE

Procedure	Results of all collaborators			Results of collaborators within ±20% of 1.78 μg/g [2]	
	Range assay results	No. collab.	Mean ±S.E. of assay results	No. collab.	Mean ±S.E. of assay results
	μg/g		μg/g		μg/g
Proposed method					
1.5 g sample					
autoclave	1.45–2.46	17	1.88±0.06	15	1.82±0.05
hot water	1.46–2.06	8	1.84±0.08	8	1.84±0.08
3.0 g sample					
autoclave	1.73–2.25	17	1.96±0.04	14	1.90±0.03
hot water	1.45–1.98	8	1.80±0.06	8	1.80±0.06
Collab. own methods					
autoclave	1.43–2.65	12	2.12±0.10	5	1.80±0.11
hot water	1.31–2.16	8	1.91±0.10	6	1.96±0.06
Microbiological method	1.55–2.69	11	2.03±0.10	7	1.83±0.08

[1] Calculated riboflavin content = 1.78 μg/g.
[2] 1.42 to 2.14 μg/g.

is slightly lower than that obtained on the 1.5 g sample, the difference is not statistically significant.

Method of Extraction. The data indicate that the extract may be prepared either by the use of an autoclave or a hot water bath without significant influence on the results. This was equally true for the proposed procedure and for the collaborators' own procedures.

BREAD SAMPLE

As expected, the analysis of the bread sample proved more difficult than analysis of the flour sample. The bread sample was calculated [7]

[7] 18.466 kg flour (0.45 μg/g), 369 g yeast (14.5 μg/g), and 20.569 mg riboflavin added to yield 34.23 mg riboflavin per 19.25 kg of air-dried bread which thus contained 1.78 μg riboflavin per gram.

to contain 1.78 μg riboflavin per gram. The data submitted are summarized in Table II.

Range of Values. In two of the categories in the table (proposed procedure, hot water extract of both the 1.5 and 3.0 g samples) all of the results were within ±20% of the calculated riboflavin content of the sample. Of the results in the remaining five categories, only one (collaborators' own procedures, hot water extraction) was below 1.42 μg riboflavin per gram (80% of 1.78 μg/g), while there were 17 results above 2.14 μg/g (120% of 1.78 μg/g). The distribution of these 17 is apparent by inspection of the table, i.e., by comparison of the number of collaborators in each category.

The data indicate that the proposed procedure, which differed to varying extents from the collaborators' own procedures, is at least as good as the various methods in use. The means of all the results obtained by the proposed procedure generally agree with the calculated riboflavin content of the bread samples slightly better than observed by Andrews (1944) in his study in spite of the use of a more difficult sample (less riboflavin present). This probably simply reflects the added experience of the collaborators.

Influence of the Size of Sample. As is the case with the flour sample, the mean riboflavin contents of the 1.5 and 3.0 samples are not significantly different for the collaborators whose results are within ±20% of the calculated content.

Method of Extraction. The results, as judged by the means of the 80 to 120% group, do not favor either method of extraction.

Comparison of the Proposed Procedure with Collaborators' Own Procedures. The results demonstrate strikingly that the proposed procedure is at least as good as the collaborators' own procedures. This is borne out not only in the means of all the collaborators but also by the number of collaborators who reported a result within ±20% of the calculated content. Since in several instances the collaborators' own procedures differed markedly from the one proposed, the latter may have been compared under slightly disadvantageous circumstances. The proposed procedure thus appears to have the practicality essential for general use.

Microbiological Procedure. It was observed that the U.S.P. procedure in the hands of six of the reporting laboratories tended to be slightly high, and four of the results were more than 20% above the calculated content. While the microbiological procedure is necessarily required to supply an independent check, these data demonstrate that its limitations are not less than those which operate in the fluorometric procedure.

General Observations

Size of Sample. The results by the proposed procedure appear to favor the smaller sample. It is interesting to note, however, that five of the collaborators reported that they regularly use 5.0 or 6.0 g samples with good results. The results submitted by these laboratories on both the flour and bread samples of this study were within the 80 to 120% group. This writer is not prepared to recommend that such large samples be used for test but does believe that if no significant objection to the use of a 3.0 g sample can be demonstrated, it is to be preferred to one of a smaller size (1.0 to 2.0 g). The accuracy of any fluorometric riboflavin assay is limited by the sensitivity of the fluorometers currently available. Until more sensitive instruments are developed it appears to the writer that the larger sample offers one means of reducing the effect of this limitation.

The practice in some laboratories of assaying 1.0 to 2.0 g samples had led them to continue the use of Florisil to keep down the volume of the riboflavin extract. That this often leads to inaccurate results is indicated by the fact that six of the collaborators using Florisil in their own procedures reported results more than 20% too high for the bread sample. This is in accord with the observations of Andrews (1944) as well as those of Hoffer, Alcock, and Geddes (1944) regarding the lack of reliable results when Florisil is used. This evidence also suggests it would be better to use 3.0 g sample for extraction to increase the riboflavin concentration of the extract.

Instruments. No correlation between results and instruments used could be demonstrated. It is well known, of course, that some of the instruments currently used are not sufficiently sensitive to be satisfactory, but present conditions make it difficult to correct this situation immediately. In all, five industrial fluorometers served in this study, though most laboratories favor one of the recently developed models.

Summary

The results of the 1944–45 collaborative study on the fluorometric estimation of riboflavin in breads and flours were gratifying both with regard to the excellent response in obtaining a representative number of collaborators and with regard to the quality of results submitted by the participating laboratories.

The results permitted a comparison of three assay methods as applied to flour containing added riboflavin and to bread baked from that flour, a comparison of the results of the assays on 1.5 and 3.0 g samples by the proposed procedure, and, somewhat indirectly, a comparison of two methods of extraction of the vitamin from the samples.

The procedure developed by this Association compared favorably with those currently used in the collaborating laboratories. As judged by the number of collaborators whose results fell within 80 to 120% of the calculated riboflavin content of the bread sample (1.78 μg/g), the proposed procedure appeared to have the requisite practicality and precision. Of the 50 results on the bread sample assayed by the proposed procedure, 45 were within ±20% of the calculated riboflavin content of the sample, while only 11 of the 20 results by the collaborators' own fluorometric procedures were within this range. Of the 81 results by all procedures 71 were within ±20% of the calculated riboflavin content of the flour sample (1.56 μg/g). This was in accord with previous collaborative results which demonstrated agreement among collaborators on assays of flour samples.

The results on the 1.5 and 3.0 g samples were not significantly different. Since a larger sample is to be preferred on general grounds, the 3.0 g amount appears to be a reasonable compromise.

Either autoclaving or hot water extraction may be used to extract the vitamin.

Acknowledgments

The writer wishes to express his appreciation to Mrs. Mary Lenz for the statistical treatment of the data and to the following who participated in the collaborative study: G. W. Agee, Barrow-Agee Laboratories, Memphis, Tenn.; H. J. Alleman, The Kroger Food Foundation, Cincinnati, Ohio; J. S. Andrews, General Mills, Minneapolis, Minn.; H. J. Cannon, Laboratory of Vitamin Technology, Chicago, Ill.; L. J. Daniel, Cornell University, Ithaca, N. Y.; W. F. Geddes and F. C. Olson, University of Minnesota, St. Paul, Minn.; D. Glick, Russell-Miller Milling Company, Minneapolis, Minn.; R. L. Gray, Lucidol Corp., Buffalo, N. Y.; L. W. Haas, The W. E. Long Company, Chicago, Ill.; A. Hoffer, Purity Flour Mills, Winnipeg, Man.; C. Hoffman, Ward Baking Company, New York, N. Y.; M. Johnson, General Foods Corp., Hoboken, N. J.; A. R. Kemmerer, Texas Agr. Expt. Sta., College Station, Texas; A. J. King, Fisher Flouring Mills Company, Seattle, Wash.; G. J. Laemmle, The Wheatena Corp., Rahway, N. J.; J. H. Lanning, Continental Baking Company, Jamaica, N. Y.; H. W. Loy, Jr., F. D. A., Washington, D. C.; D. Melnick, Food Research Laboratories, Long Island City, N. Y.; G. D. Miller, Omaha Grain Exchange, Omaha, Nebr.; J. H. Panton and P. K. Dederick, Winthrop Chemical Co., Rensselaer, N. Y.; J. Rosin, Merck and Co., Rahway, N. J.; S. H. Rubin, Hoffmann-LaRoche, Nutley, N. J.; L. T. Saletan, Schwarz Laboratories, New York, N. Y.; H. C. Schaefer, Ralston Purina Co., St. Louis, Mo.; H. K. Steele, The Fleischmann Laboratories, New York, N. Y.; B. Titlow, Department of Agriculture, Columbus, Ohio; H. B. Wigman, Mellon Institute, Pittsburgh, Pa.; L. Feinstein, W. F. A., Beltsville, Md.; F. M. Strong, University of Wisconsin, Madison, Wis.

Literature Cited

Andrews, J. S.
1943 A collaborative study of riboflavin assay methods. Cereal Chem. **20**: 3–23.
1943a Report of 1942–43 methods of analysis subcommittee on riboflavin assay. Cereal Chem. **20**: 613–625.
1944 Report of the 1943–44 methods of analysis subcommittee on riboflavin assay. Cereal Chem. **21**: 398–407.
Hoffer, A., Alcock, A. W., and Geddes, W. F.
1944 Some factors affecting the determination of riboflavin by the fluorometric method. Cereal Chem. **21**: 515–523.

BOOK REVIEW

The Estimation of Vitamin A. By N. T. Gridgeman. 76 pp. Lever Brothers and Unilever Limited, London. 1944. (Distributed free to interested scientists by Lever Brothers Co., Cambridge, Mass.)

This brochure presents a critical summary of present methods and conventions used for estimating vitamin A as a basis for the author's contention that a new metrology should be adopted for this vitamin.

The author points out first that while specifications for the vitamin A content of commercial products are invariably written in terms of biological units, the experimental difficulties involved in bioassays have led to substitution of the simpler spectrophotometric techniques for routine work. He then summarizes the available data for the conversion factor $\left(\text{C.F.} = \frac{\text{Units per gram}}{\text{Extinction Coefficient at 325 m}\mu}\right)$, pointing out the variability of published results and calculating the limits of error for each series of data. In considering the sources of variation in correction factors, he discusses in some detail the factors that influence the accuracy of the spectrophotometric and bioassay methods. He then traces the historical developments which have culminated in the present official designation of the International Unit as 0.6 μg of the standard β-carotene and points out certain discrepancies in results obtained for the potencies of the U.S.P. Reference Cod-liver Oils. The fundamental suitability of a carotene standard is questioned in view of abundant evidence that the utilization of carotene varies with species and diet.

The potency of pure vitamin A is discussed and the available data are summarized and criticized in the light of theories of the mechanism by which carotene is converted to vitamin A *in vivo*. Factors affecting the stability of vitamin A in oils are discussed briefly and some attention is devoted to the utilization of vitamin A congeners and esters.

The entire summary lends strong support to the conclusion that "the current bioassay of vitamin A, although an important research instrument, is to all intents and purposes valueless as a method of routine estimation" because of (1) large normal animal variations and (2) the fact that "the bioassay is dependent on biological standards that are patently unsatisfactory." In view of these difficulties, the author's proposal of a new unit of vitamin A based on the absorption properties of the vitamin itself certainly will merit careful consideration at future conferences on vitamin A standardization.

Several interesting and stimulating "pertinent research problems" are suggested, among which are: (1) further study of the absorption properties of vitamin A-potent materials including those of the antimony trichloride reaction products, (2) development of techniques for estimation of vitamin A in low-potency materials, and (3) study of the biological potencies (perhaps based on some other criterion than rat growth) of various vitamin A preparations in relation to their extinction coefficients.

Methods for making bioassays and of treating them statistically are presented in some detail in a nine page appendix. The bibliography includes 175 references.

ROBERT JENNESS,
Division of Agr. Biochemistry,
University of Minnesota,
St. Paul 8, Minnesota

TWO BOOKLETS YOU'LL SURELY WANT TO READ...

1.
"HOW BREAD AND B-E-T-S GOT TOGETHER"

2.
"THE STORY OF VEXTRAM"

Sent FREE and POSTPAID—request your copies TODAY!

In addition to "B-E-T-S" for bread-enrichment, "VEXTRAM" for flour-enrichment, and RIBOFLAVIN MIXTURE for mixed feeds, WINTHROP also has available the following nutrients:

CRYSTALLINE **VITAMIN B_1** — CRYSTALLINE **VITAMIN C**

d-ico-ASCORBIC ACID

RIBOFLAVIN — **VITAMIN B_6**

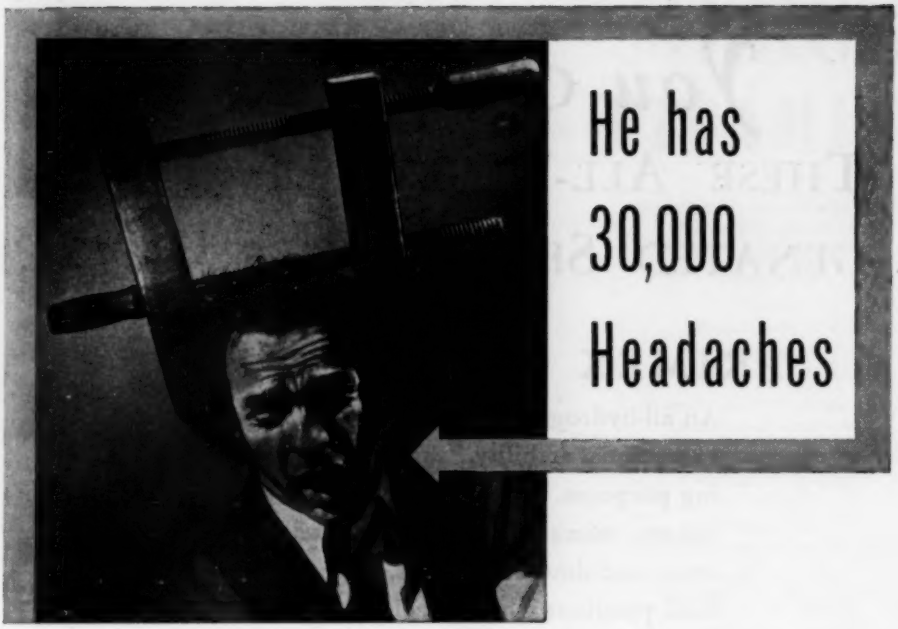
CALCIUM PANTOTHENATE

Stocked for quick delivery at New York, Chicago, Kansas City, (Mo.), Denver, San Francisco, Portland, (Ore.), Dallas and Atlanta.

HERE'S reading material that's a *must* for you, if you want to keep abreast of "goings-on"—past and present—in the bread and flour-enrichment fields. Send today for your copies of these two interesting booklets. They tell the story of Winthrop Chemical Company's bread and flour-enrichment mixtures — "B-E-T-S" and "VEXTRAM"—and highlight the history of enrichment in the baking and milling fields. Direct your request to *Special Markets Division*, Winthrop Chemical Company, Inc. at address shown below.

Address Inquiries to—

Special Markets Division
WINTHROP CHEMICAL CO., INC.
170 Varick Street, New York 13, N.Y.



He has 30,000 Headaches

These are strenuous days for your laboratory supply dealer. No matter what happens he is always in the middle—squeezed between your needs and the manufacturer's ability to supply them.

Every day he faces 30,000 potential headaches—for that is about the average number of items he tries to carry in stock. Some of these are in daily demand. Others are called for only infrequently. Yet when you want them—YOU WANT THEM.

Confronted with shortages on every hand, perplexed by priorities and faced with the loss of employees to the Armed Services, your dealer carries on valiantly. Corning salutes him and bespeaks for him from you as much consideration and cooperation as you can possibly give.

CORNING GLASS WORKS • CORNING, NEW YORK

PYREX BALANCED FOR ALL-AROUND USE brand LABORATORY GLASSWARE

CORNING means Research in Glass

RIGHT ON THE LABEL

...to help sell these popular foods!

A Breakfast Cereal Says . . .
"Contains more Calcium, Phosphorus and Iron than whole wheat flour . . . and as much Vitamin B_1. Calcium and Phosphorus are useful in the normal formation of bones and teeth. Iron is useful in the normal formation of hemoglobin."

A Baby Cereal Says . . .
"Enriched with iron . . . to help build sturdy little bodies . . . One ounce supplies the following proportions of the minimum daily requirements . . . for both infants and young children, 1¼ times the iron . . . 1/5 of the calcium and phosphorus."

A Milk Amplifier Says . . .
"Amplifies the food value of milk by adding Iron and Vitamin D. Four teaspoonfuls, the amount recommended per quart of milk, supplies the full minimum daily requirement of Iron and Vitamin D."

A Candy Bar Says . . .
"Supplies twelve vitamins and 8 minerals including calcium, phosphorus, iron, iodine, copper, manganese, magnesium, and zinc."

Facts such as these . . . printed right on the label . . . do a real selling job for these popular foods. And no wonder! According to recent reports,* 2 out of every 3 persons are *not* getting enough minerals and vitamins in their food. We are constantly being reminded of mineral and vitamin deficiencies in magazines, newspapers and over the radio.

It is only natural that housewives will prefer . . . and buy . . . products such as these that provide the extra nourishment authorities agree is so essential to good health!

Victor mineral supplements can help sell your product, too. They can readily be incorporated in bread, flour, cereal, soup, candy and many other staple foods. For over 40 years Victor Chemical Works has specialized in the production of food grade phosphates and mineral supplements. Our technical staff will be glad to offer any assistance possible.

**See Journal of the American Dietetic Association, Dec. 1944.*

VICTOR *mineral supplements:* Monocalcium Phosphate . . . Dicalcium Phosphate . . . Tricalcium Phosphate . . . Calcium Pyrophosphate . . . Sodium Iron Pyrophosphate . . . Ferric Orthophosphate . . . Ferric Pyrophosphate

Experimental samples on request

VICTOR *Chemical Works*

WORLD'S LARGEST MANUFACTURERS OF FOOD PHOSPHATES

141 West Jackson Boulevard, Chicago 4, Illinois

GET FAST, CONTROLLED DRYING

for Flax and Soy Tests

with

DESPATCH BUILT

LABORATORY OVENS

***WRITE TODAY** for this helpful new Bulletin 105-CC describing Despatch Laboratory Ovens. Fully illustrated; printed in 2 colors.*

There's no faster, safer, more accurate method of drying flax or soy samples than to run them through a forced-draft *Despatch* Laboratory Oven! Every test gets optimum heat application, accurately controlled.

You can run *oil content determinations* (drying before grinding and re-drying to eliminate solvent), *moisture content tests, constant weight analyses* and scores of other tests with equal ease and accuracy.

FEATURES: Fast heat recovery, 2–8 min. after loading cold samples • Thermostat sensitive to ±1°C. • Wide-range flexibility, 35° to 260°C. • 5 year guarantee of heat elements • Convenient sizes, 13" x 13" x 13" to 37" x 25" x 37"; electric or gas models.

Ask your Dealer for Details

or write direct

DESPATCH OVEN COMPANY
MINNEAPOLIS 14
MINNESOTA, U.S.A.